IN VITRO ANTIMICROBIAL ACTIVITY AND TOXICITY OF SELECTED MEDICINAL PLANTS USED TO MANAGE BACTERIAL GASTROENTERITIS IN MERU COUNTY, KENYA

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NOVEMBER, 2022

DECLARATION

I declare that this is my original work and has never been submitted for award of any other degree in this or any other University

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DEDICATION

This thesis is dedicated to my family members for the financial support they gave me since the beginning of this study.

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ACRONYMS AND ABBREVIATIONS

AAS	Atomic absorption spectrometry
Aids	Acquired Immunodeficiency Syndrome
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
API	Analytical profile index
As	Arsenic
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
BUN	Blood urea nitrogen
bw	Body weight
Ca	Calcium
Cfu	Colony forming units
CK	Creatinine kinase
CLSI	Clinical Laboratory Standard Institute
Cr	Chromium
Cu	Copper
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
Fe	Iron
g	gram
GGT	Gama glutamyltransferase
g/kg	Grams per kilogram
H_2O_2	Hydro-peroxicide
Hb	Hemoglobin
HDL	High Density lipoprotein
HE	Hektoen enteric agar
HNO_3	Nitric acid
Hr	Hour
K	Potassium
KEMRI	Kenya Medical Research Institute
kg	kilogram

LDH	Lactate dehydrogenase
LDL	Low Density lipoprotein
MBC	Minimum bactericidal concentration
MCV	Mean cell volume
mg/kg	Milligrams per Kilograms
MHB	Muller Hinton Broth
MIC	Minimum inhibitory concentration
ml	milliliter
Mn	Manganese
ORT	Oral rehydration therapy
MCHC	Mean cell hemoglobin concentration
PCR	Polymerase chain reaction
MCHC	Mean cell hemoglobin concentration
PCV	Packed cell volume
Mg	Magnesium
PPM	Parts per million
RBC	Red Blood Cells
SD	Standard deviation
SS	Salmonella Shigella agar
WBC	White Blood Cells
WHO	World Health Organization
XLD	Xylose Lysine Deoxycolate Agar

ABSTRACT

Herbal drugs have been used in management of bacteria causing bacterial gastroenteritis without scientific evaluation on their antimicrobial activity and toxicity. This research was designated to evaluate *in vitro* activities of methanol extracts of *Aloe deserti*, *Zanthoxylum chalybeum* and *Zanthoxylum usambarense* and assess their possible toxicity using the mice models. The crude extracts were evaluated on their activities against Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430 and *Shigella dysenteriae* ATCC 13313) and Gram-positive bacteria (*Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29912) using the Disk Diffusion Method, Minimum Inhibitory Concentration (MICs), Minimum Bactericidal Concentration (MBCs) and Time Kill studies. Methanol extracts of *Z. usambarense* showed strong susceptibility against five tested bacteria (*B. cereus* ATCC 10876, *B. subtilis* ATCC 6633, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29912, *E. coli* ATCC 25922) and the other two bacteria had weak susceptibility (*S. typhi* ATCC 19430, *S. dysenteriae* ATCC 13313). Methanol extract of *Z. chalybeum* was active only to two Gram-positive bacteria (*B. subtilis* ATCC6633, *B. cereus* ATCC10876). *Aloe deserti* methanol extracts were completely inactive against all the tested bacteria. Only *Z. usambarense* extracts was evaluated on MICs, MBCs and Time Kill because of its high bioactivities. The MICs results gave good low MICs of 12.5mg/ml and 50mg/ml. The MBCs were as low as 12.5mg/ml while the highest being 100mg/ml. MBC/MIC values indicated good activities of 1.0 (bactericidal activities). Time Kill Assay was determined by plate count technique and analyzed by % kill or growth of viable colonies. The herbal drug displayed bacteriostatic activities toward test bacteria (*B. cereus* ATCC 10876, *B. subtilis* ATCC 6633) at 0.5×MIC concentration after 24 hr exposure. Methanol extract of *Z. usambarense* displayed time dependent killing kinetics that ranged between100 and 96 cfu/ml for *S. aureus* ATCC 29213, 100 and 98 cfu/ml for *E. feacalis* ATCC 29912, and 131 and 96 cfu/ml for *E. coli* ATCC 25922. The herbal drug was rapidly bactericidal at 1×MIC concentration achieving 99.9 % elimination of *B. cereus* ATCC 10876 and *B. subtilis* ATCC 6633 and at 2×MIC killing completely (99.9 %) *B. cereus* ATCC 10876, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 29213 after 8 hr exposure except *E. feacalis* ATCC 29912 and *E. coli* ATCC 25922 displaying bacteriostatic activities. Toxicity in mice was assessed by administering extracts orally and intraperitoneally at 450, 670 and 1000 mg/kg body weight after 4 weeks by noting changes in behavior, body and organ weight, hematological and biochemical parameters. The reduced growth rate, organ weight and increased levels hematological and biochemical parameters indicated slight toxicity of plant extracts. The same dose in both routes increased the liver and spleen, and decreased the testis weight, and increased the hemoglobin levels, packed cell volume and platelets; increased the activity of aspartate aminotransferase and lactate dehydrogenase, and decreased the activity of alkaline phosphatase, gamma-glut amyl transferase, and creatine kinase slightly injured the spleen, kidney and liver organs. Toxicity studies confirmed the safety of plant extracts in both routes. The phytochemical analysis results showed the presence of alkaloids, tannins, glycosides, flavonoids, terpenoids and anthraquinones. Analysis of elements such as Mg, K, Ca, Mn, Fe, Zn, Pb, Cr, Sr, Cu, As, V, and Hg were done using AAS and results showed presence of metals at varying concentrations. The measured mineral did not cause toxicity because their levels were below the recommended daily allowance. In conclusion, the observed antimicrobial activity and slight toxicity could be associated with phytochemicals and minerals present in extracts. Results from this work recommend use of *Z. usambarense* extract as phyto-medicine to manage bacterial gastroenteritis. Further studies of *Z. usambarense* are recommended using higher animals.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Bacterial gastroenteritis is a disease condition caused by a large group of Gram-negative and Gram-positive enteric bacterial pathogens (Tambekar, 2010). In low-income countries or developing countries, disease causing micro-organisms such as *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Vibrio cholerae, Salmonella typhi,* and *Shigella dysenteriae* are typical causative agents of diarrheal infections in humans (Reuben, 2009). The mode of spreading is ingestion of contaminated foods with human stool and sometimes through fecal-oral route (Nahed, 2011). Bacterial gastroenteritis causes economic loss resulting in low productivity at work place due to labour shortage, increased health care costs or longer hospital stay (Nahed, 2011). The children and elderly people are at risk of mortality from bacterial ailment (gastroenteritis) because of treatment failures (Nahed, 2011). Diarrheal infections are the primary public health problems in developing countries and affects 5 billion young people every year (Reuben, 2009) and accounts for 3 to 6 million deaths annually among individuals less than 5 years old (Payne, 2004).

Bacterial infections are managed by antibiotics that kill, prevent or inhibit the multiplication of pathogenic bacteria within the human body (Sabahat, 2005). Antibiotics have been weakening in their effectiveness (becoming less effective) in treating ordinary bacterial infections (Bensode, 2012). The reason for this unfortunate performance is bacterial resistance to antibiotic drugs (Prescott, 2005). Such resistance builds up when bacteria become accustomed to and grows or multiplies in

presence of antibiotics (Abiba, 2013). This kind of resistance develops through poor infection controls in hospitals, over prescription (extensive use), misuse and overuse of antibiotics (Bensode, 2012). The improper use of antibiotics has made bacteria to evolve and develop resistance (become unyielding to drugs), resulting in emergence of untreatable diseases that threaten the basis of conventional medicine (Prescott, 2005). The increasing number of drug-resistant bacteria is one of the serious health problems as bacteria no longer responds to conventional antimicrobial drugs (Prescott, 2005). Antibiotics are now failing to do what they were designed to do, and this poses the greatest threat to humans' health today (Mosaid, 2012).

The bacteria resistance mechanism is spreading fast threatening capability to treat bacterial infectious diseases such as gastroenteritis especially in children due to failure to develop new drugs (Bensode, 2012). These infections are becoming harder and sometimes impossible to manage as humans run out of treatment options (Prescott, 2005). Another reason for antibiotics resistance is lack of capacity by health facilities in developing countries to do culture or sensitivity test to identify the exact bacteria causing infections leading to wrong medication (Prescott, 2005).

The resistance of bacteria to drugs has necessitated survey for alternative medicines especially from plants aiming to reduce resistant bacterial pathogens in humans' population (Tambekar, 2010). The effort to combat resistance, re-emergence and spread of antibiotics resistance adds urgency to shift medicinal care from orthodox to herbal drugs (Otimenyin, 2008) and now new effective therapeutics are imperative (Mosaid, 2012). Herbal drugs, in which plant extracts are used as health-giving substance, is preferred because of their accessibility, affordability, assumed non-toxic

herbal substance and their fewer side effects (Tambekar, 2010). World Health Organization (WHO) has approximated 65% - 80% of total human population use herbal drugs for their therapeutic needs (Mosaid, 2012).

1.2 Statement of the problem

The increased resistant bacteria, emergence of bacteria with reduced susceptibility to antibiotics, toxic effects of conventional drugs, treatment failures/failures to develop new drugs and high health care costs has forced man to research on alternative medicine more so from medicinal plants (Poole, 2001). The toxicity of herbal medicine is an area of interest among the health personnel's who distrust herbal therapeutics because of lack of proof of safety (Adenike, 2006). This research is anticipated to evaluate *in vitro* antimicrobial activity and toxicity of methanol extract of *Zanthoxylum usambarense, Zanthoxylum chalybeum and Aloe deserti* plants used to manage enteric bacterial infections (caused by bacteria such as *B. cereus, S. aureus, E. faecalis, E. coli, b. subtilis, S. typhi* and *S. dysenteriae*) (Baby, 2013) that commonly lead to bacterial gastroenteritis. This is based on knowledge passed by Meru communities from generation to generation for many years.

1.3 Justification of the study

Emerging resistant bacteria causes untreatable bacterial infections that lead to unbearable human suffering (Reuben, 2009). There are also worries on the toxicity of the existing antibiotic drugs (Baby, 2013). According to Abiba (2013), herbal drugs have been used to manage bacterial infections to avoid the emergency of resistant bacteria. The use of herbal drugs has improved human health and life expectancy (Mariita, 2010). The phyto-antimicrobial agents are of great importance especially

during the search for new drugs targeting human bacterial pathogens. The decoction of *Zanthoxylum usambarense* (barks)*, Zanthoxylum chalybeum* (barks) and *Aloe deserti* (leaves) have been used by local communities in Meru County to manage diarrhea or bacterial gastroenteritis. In addition, there is no proof of *in vitro* antimicrobial bioassay and *in vivo* toxicity studies of *Z. usambarense, Z. chalybeum* and *A. deserti* extracts. This is the basis of this study.

1.4 Research questions

i) Do methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* possess antimicrobial activity?

ii) Are the methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* safe when used in experimental mice model?

iii) Do methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* possess phytochemicals and minerals associated with antimicrobial activity?

1.5 Null hypotheses

i) Methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* plants does not have any antimicrobial activity.

ii) Methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* plants administration have no toxicity in mice model.

iii) The phytochemicals and minerals in methanol extract of *Z. usambarense, Z. chalybeum a*nd *A. deserti* do not contribute to the antibacterial activity.

1.6 Objectives of the study

1.6.1 General objectives

To evaluate *in vitro* antimicrobial activity and *in vivo* toxicity of methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti*.

1.6.2 Specific objectives

i) To assess antimicrobial activity of *Z. usambarense, Z. chalybeum* and *A. deserti* methanol extracts.

ii) To examine the toxicity of *Z. usambarense, Z. chalybeum* and *A. deserti* methanol extracts.

iii) To determine phytochemicals and mineral compositions of methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti*.

1.7 Significance of the study

The acquired knowledge on phytochemical activities against pathogens is expected to stimulate research and documentation of medicinal plants. Besides, the active compounds screened from medicinal plants extracts will be proposed for the development of drugs, capsulation and tableting for commercial purposes by institute of alternative medicine. Information obtained will be used to encourage the public to propagate medicinal plants for future exploitation for their medicinal values. Herbal medical practitioners seeking new precedence to develop advanced drugs will use these methods to screen herbal drugs and confirm their efficacy. In addition, the study avails information on alternative therapy for the management of bacterial gastroenteritis. The findings will be used as a baseline study for other researchers working on pathogenic bacteria management and reference for their studies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Enteric bacterial pathogens

The common bowel pathogens that cause bacterial gastroenteritis in developing countries are *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Bacillus subtilis, Salmonella typhi, Vibrio choleraea* and *Shigella dysenteriae* (Reuben, 2009). Sometimes, people refer to gastroenteritis as 'stomach flu' which is characterized by symptoms such as diarrhea (that may contain blood, mucus or pus) (Nahed, 2011). Diarrhea (loose or watery stools) is an unusual frequency of bowel action which is the most dangerous bacterial gastrointestinal disorder in children (Reuben, 2009) and elderly or aged people (Nahed, 2011).

2.2 Causes of bacterial gastroenteritis

2.2.1 Characteristics of Gram-positive bacteria

2.2.1.1 *Staphylococcus aureus*

Staphylococcus aureus is a facultative anaerobic Gram-positive spherical bacterium belonging to the family Staphylococcaceae (Kaigongi, 2014). *Staphylococcus* is a non-spore forming bacterium that occurs in clusters and reproduces asexually by binary fission (Abiba, 2013). The pathogen is mostly found on mucous membrane of human beings. *Staphylococcus* infection is associated with consumption of contaminated foods (India, 2015). *Staphylococcus aureus* is an opportunistic pathogen and releases staphylococcal enterotoxin that causes staph infections such as enterotoxin food poisoning or gastroenteritis characterized by watery diarrhea, electrolyte disproportion and loss of body fluids (Haque, 2011). *Staphylococcus*

diseases are difficult to control because of acquired resistant to antibiotics such as β-lactam agents (Oyetuuji, 2012).

2.2.1.2 *Bacillus cereus*

Bacillus cereus is a Gram-positive, facultative anaerobic, rod shaped motile encapsulated bacillus, belonging to the family Bacillaceae (Abiba, 2013). The pathogen produces spores that are resistant to unfavorable conditions. *Bacillus* is widely dispersed in the environment including mucous membrane of a health human beings (Kaigongi, 2014). *Bacillus* infection is associated with consumption of contaminated foods and water. The gastroenteritis results from enterotoxin produced by the organisms in immunocompromised patients (Abiba, 2013) and gives rise to symptoms such as abdominal pain and watery diarrhea (bacillus gastroenteritis) (Bartram, 2003). *Bacillus cereus* infections are not easy to control because of acquired resistant to β-Lactam antibiotics (Kaigongi, 2014).

2.2.1.3 *Enterococcus faecalis*

Enterococcus feacalis is a Gram-positive, non-spore forming, facultative anaerobic bacillus, belonging to the family Enterococcaceae (Franz, 2003). The pathogen settles in the gastrointestinal tracts of a person and other mammals. *Enterococcus* infection is associated with consumption of contaminated foods and water (Kavindra, 2010). *Enterococcus faecalis* is an opportunistic pathogen that releases enterotoxins that cause foodborne illness (Zhou, 2013) and gives rise to stomach flu characterized by loose stool (enterococcus gastroenteritis) (Kavindra, 2010). The pathogen exhibits decreased susceptibility to commonly used antibiotics (Franz, 2003).

2.2.1.4 *Bacillus subtilis*

Bacillus subtilis is a spore forming, motile, rod-shaped, Gram-positive, facultative anaerobic bacterium belonging to the family Bacillaceae (Hong, 2009). The pathogen is mostly found in soil and tolerates harsh environmental conditions. *Bacillus subtilis* is non-infectious but can be an opportunistic pathogen to immunocompromised HIV/Aids patients (India, 2015). Its infection is through taking foods and water contaminated by organisms. It releases enterotoxins that cause foodborne illness (Rahman and Kang, 2009) and give rise to symptoms such as vomiting, abdomen pain and diarrhea (bacillus gastroenteritis) (Hong, 2009). *B. subtillis* infections are not easy to manage because of acquired resistant to β-lactam agents (Kaigongi, 2014).

2.2.2 Characteristics of Gram-negative bacteria

2.2.2.1 *Salmonella typhi*

Salmonella typhi is a Gram-negative, motile, rod shaped, flagellated bacterium, facultative anaerobic and non-spore forming member of the family Enterobactericeae (Abiba, 2013). The pathogen is responsible for causing watery diarrhea (salmonella gastroenteritis) or typhoid fever and gives rise to symptoms such as vomiting and abdominal pain (Cooke, 2010). This pathogen mainly settles in the digestive system of humans and is eliminated together with stool of the infected individuals (Kim, 2004). *S. typhi* transmission is by ingesting food and water contaminated by organisms. The outbreak occurs due to unhygienic preparation and poor food storage (Nahed, 2011). Worldwide there is rampant spread of *S. typhi* resistance to antibiotics (WHO, 2015).

2.2.2.2 *Shigella dysenteriae*

Shigella dysenteriae is a facultative anaerobic, non-capsulated, Gram-negative bacterium belonging to the family Enterobactericeae (Niyogi, 2005). The pathogen is found only in the digestive system of human beings. *Shigellae* infection is by stool to mouth (faecal-oral way) especially in children (Abiba, 2013). It releases enterotoxins that cause dysentery (Shigellosis) and gives rise to symptoms such as vomiting, abdominal pain and bloody diarrhea. *Shigella* is capable of invading and colonizing the epithelium of intestine resulting to severe inflammatory colitis. *Shigella spp* are resistance to most antibiotics, emerging as multidrug resistant (India, 2015). Shigellosis mortality rate is high amongst children below 5 years of age and elderly people (Nahed, 2011).

2.2.2.3 *Escherichia coli*

Escherichia coli is a Gram-negative, rod-shaped bacterium, facultative anaerobic and non-spore forming member of the family Enterobactericeae (Emecar, 2010). *E. coli* usually settles in the lower part of digestive system of humans and is eliminated in the faeces of the infected individuals. *E. coli* are harmless, but a few strains such as *E. coli* 0157:H7 (enterohemorrhagic *E. coli*) causes bloody diarrhea and enterotoxigenic *E. coli* causes travellers' diarrhea (Mariita, 2010). Its transmission is by ingesting food/water contaminated by the organisms. The shiga toxin released by the pathogen is responsible for causing bloody diarrhea disease/gastroenteritis in infants and elderly persons which is indistinguishable from haemorrhagic colitis (Eryilmaz, 2010) disrupting absorptive function of intestinal mucosal cells (Abiba, 2013). *E. coli* exhibit resistance to antibiotics (Mariita, 2010).

2.3 Economic importance of enteric bacterial pathogens

In case enteric bacterial pathogen infection fails to respond to treatment, the implication is that patient suffers trauma leading to untold suffering, rise of treatment costs and general gastroenteritis management practices become more resource demanding (Umaira, 2011). The cost of treating patients' raises because of higher cost of drugs, longer period of hospitalization and increased cost of management within the healthcare systems (Mariita, 2010). The development of antibiotic drugs and supplies are cost intensive, ranging from 100$ to 350$ (Chan, 2003). In developed countries $6 billion is spent on medical services due to bacterial infections or illness, most of which causes diarrhea in humans (Umaira, 2011). The productivity of workers declines because of absenteeism in workplaces (Chan, 2003).

2.4 Laboratory diagnosis

2.4.1 Culture techniques

Stool samples are transferred onto nitro-cellulose paper and mixed with selective and differential media. Microorganisms are identified using morphology and biochemical characteristics (Abubakar, 2007).

2.4.1.1 *Salmonella* and *Shigella*

Stool samples (1 g) are inoculated into 10ml of Selenite broth for enrichment at 37°C for 18 to 24 hours (Abu, 2008). Colonies are purified by sub-culturing onto *Salmonella Shigella* (SS) and Xylose Lysine Deoxycolate agar (XLD) (Nahed, 2011) and incubated at 37°C for 18 to 24 hours. Colony morphology of *S. typhi* appears on SS agar and XLD agar as colorless colonies with black center. The *S. dysenteriae* on SS agar appear as colorless while on XLD agar colonies appear as transparent red

(Abu, 2008). The analytical profile index (API) is used for confirmation of *S. typhi* and *S. dysenteriae* (Nahed, 2011).

2.4.1.2 *Escherichia coli*

One gram of stool samples is inoculated into 10ml of MacConkey broth for enrichment at 37°C for 18 to 24 hours (Paresh, 2013). The enrichment is streaked onto MacConkey agar and incubated for 24 hours at 37°C (Zhou, 2002). Pink colored colonies are purified by sub-culturing on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 18 to 24 hours (Paresh, 2013). Colony morphology of *E. coli* on EMB agar appears as greenish metallic (Zhou, 2002). The analytical profile index (API) is used for confirmation of *E. coli* (Paresh, 2013).

2.4.1.3 *Staphylococcus aureus*

Stool samples (1g) are inoculated into 10ml of nutrient broth for enrichment at 37°C for 18 to 24 hours (Nayak, 2013). The enrichment is streaked onto mannitor salt agar (MSA) and incubated for 24 hours at 37°C (Ali, 2007). Appearance of black colonies on MSA surrounded by clear halos indicates *S. aureus* (Nayak, 2013). The catalase test is used for confirmation of *S. aureus* (Ali, 2007).

2.4.1.4 *Bacillus* species

Stool samples (1 g) are inoculated into 10ml of nutrient broth for enrichment at 37°C for 18 to 24 hours with vigorous shaking (Wafula, 2014; Chang, 2018). Colonies are purified by sub-culturing *B. cereus* onto mannitol-egg-yolk-polymxin (MYP) agar (Wafula, 2014) and *B. subtillis* on nutrient agar (NA) (Chang, 2018) and incubated at 37°C for 18 to 24 hours. Appearance of *B. cereus* on MYP agar is pink color

surrounded by a halo (Wafula, 2014). Appearance of *B. subtillis* on NA is yellow color with jagged edges (Chang, 2018). The catalase test is used for confirmation of *B. cereus* (Wafula, 2014) and *B. subtillis* (Chang, 2018).

2.4.1.5 *Enterococcus faecalis*

Stool samples (1g) are inoculated into 10ml of nutrient broth for enrichment at 37℃ for 18 to 24 hours (Ali, 2007). The enrichment is streaked onto Bile Esculin (BE) agar and incubated for 48 hours at 35℃ (Ali, 2007). Appearance of *E. faecalis* on BE is dark brown or black in color. The catalase test is used for confirmation of *E. faecalis* (Mureithi, 2013).

2.4.2 Rapid diagnosis

Rapid diagnosis involves novel testing procedures for faster detection of bacterial pathogens. This is where expeditious remedy is essential to avert kidney and liver injury. The protocols developed are molecular tools (variety of assays) (Zhou, 2013) such as nucleic acid-based methods (DNA probes/Gene encoding, hybridization and PCR by Pulsed Field Gel Electrophoresis), antibody-based tests (ELISA, RPLA and IMS) and physiochemical analyzers that measure pathogens metabolites. These assays are complicated but have excellent sensitivity (Nahed, 2011).

2.5 Management of bacterial gastroenteritis

2.5.1 Oral rehydration therapy

The treatment of bacterial gastroenteritis involves replacement of fluids and electrolyte lost orally (Tarja, 2000). Oral rehydration solution (ORS) or electrolyte solutions (Made by dissolving sodium salts) prevent dehydration in diarrheal patients

(Okebe, 2008). Use of increased amounts of fluid together with continued feeding on balanced diets prevents dehydration and hypernatremia (Tarja, 2000).

2.5.2 Antidiarrheal drugs

Antidiarrheal drugs such as cholestyramine (activated charcoal) solidifies loose stools and/or reduces stool volume and shorten the duration of diarrhea in patients (Igboeme, 2018). These drugs have no advantages in correcting dehydration especially to rehydrated patient but reduce defecation frequency of watery stool (Okebe, 2008). Antidiarrheal drugs cause serious complication and increase the cost of curing gastroenteritis (McQuain, 2004).

2.5.3 Anti-motility drugs

The anti-motility standard drugs such as atropine sulphate and Loperamide are used to control severe diarrhea to moderate diarrhea in case there is prolonged diarrheal illness in humans (McQuain, 2004). It reduces watery or bloody stool (solidifies stool) and shortens the duration of diarrhea when used together with oral rehydration therapy (McQuain, 2004). On the other hand, Loperamide cause drowsiness (sleepy) or somnolence in humans and worsens dehydration due to pooling of antidiarrheal stools in paralyzed gut or intestines. It is not recommended for use in children, elderly or aged people or people who have bloody diarrhea (Okebe, 2008).

2.5.4 Antimicrobial therapy

Antimicrobial drugs are given only selectively in cases where their use is commonly accepted and beneficial (Tarja, 2000). The causative agent should be known and antibiotic sensitivity determined for suitable medication (Tarja, 2000). *Staphylococcus*

and *Bacillus* enteritis are treated with azithromycin together with antidiarrheal drugs, *Salmonellosis* with fluoroquinolone and ceftriaxone, Shigellosis with ciprofloxacin and ceftriaxone and *Escherichia* diarrhea with tetracycline and ciprofloxacin (Tarja, 2000). Unfortunately, emergences of drug resistance microbes and drug toxicities (toxic effect) have complicated the empirical therapy of bacterial gastroenteritis or enterocolitis in humans (Bensode, 2012).

2.6 Resistance of bacteria against conventional antibiotics

The extensive use, misuse, overuse and self-medication of synthetic antibiotics in many developing countries have resulted to re-emergence of drug-resistant bacteria (Taneja, 2004). The dug-resistant microbes and drug toxicities are the greatest public health problems (Nahed, 2011). The resistance of bacteria to antibiotics is so serious that unless concerted efforts are taken, there is danger of going back to pre-antibiotic age (WHO, 2021). This kind of resistance happens when bacteria change and become unyielding to drugs used to treat the infections they cause to humans (WHO, 2021). That is, antibiotics forces microbes to either die or adapt through various mechanisms such as single point mutations in *E. coli*, modifying drug-binding site and loss of cell permeability, for example, penicillin failing to penetrate cell membrane of gram-negative bacteria (Maame, 2009).

In other cases, bacteria secrete enzyme that inactivate or destroys antibiotics, for example, β-lactamase enzymes and others secrete metabolites which bloc or antagonize drugs working mechanisms (Brigitte, 2002). The bacteria mutate, adapt and finally survive (become resistant) passing genes for resistance, to new bacteria replicants (Brigitte, 2002). This problem is compounded by lack of new antibiotics

that can kill bacteria to avoid the emergence and re-emergence of resistance bacteria (Bensode, 2012). The re-emergence of resistant bacteria necessitates the search for alternative drugs more so from plants to combat bacterial infections in humans (Maame, 2009).

2.7 Alternative therapy

Herbal medicines have great potential as antimicrobial agents against resistant bacteria pathogens (India, 2015). The use of phytomedicines such as benzoin extracted from *Styrax tonkinensis* for intestinal therapeutics (Abiba, 2013), remoxe isolated from *Ajuga remota* for malarial treatment (Kuria, 2001) and other herbal drugs such as emetine, quinine and berberine (Gurdeep, 2005) are enjoying great popularity. The discoveries of plant generated drugs have made a lot of contribution to humans health-giving and their wellbeing in management of diseases caused by drugs resistant bacteria (Njoroge, 2010).

2.8 Advantages of using herbal medicines

Herbal medicines have enormous therapeutic potential in developing countries (Tambekar, 2010). They are assumed to be non-toxic and active in management of infectious illness and mitigate any responses that are often linked with conventional antimicrobials (India, 2015). The herbal drugs are easily attainable and affordable by humble families especially where conventional antibiotics are too expensive to the local populations in developing countries (Njoroge, 2010). Because of antibiotics toxicity and microbes' resistance against antibiotics, much scrutiny has been put to evaluate active phytochemicals and this has led to search for herbal medicines with the goal to overcome the above-mentioned disadvantages (Njoroge, 2010).

2.9 Antimicrobial activities of herbal medicines

The activities of widely used herbal drugs against pathogenic bacteria have been documented by various reports of different researchers in the world since the earliest days of mankind (Nguen, 2015). The acetone stem extracts of *Dichrostachyus cinerea* plant have been validated to be active against *Escherichia coli*, causal organism of diarrheal diseases (Abiba, 2013). The methanol extracts of *Momordica balsamina* plant against *Staphylococcus aureus, Bacillus subtillis* (Gram-positive), *Escherichia coli* and *Salmonella typhi* (Gram-negative) bacteria have been noted to have antibacterial activity (Sunday, 2008).

Many herbal extracts have good antimicrobial activity against Gram-positive than Gram-negative bacteria. This is in agreement with previous reports by other workers (India, 2015). Antimicrobial activity is associated with the ability of drugs to penetrate the cell wall of the Gram-positive bacteria (Abiba, 2013). The Gram-negative bacteria have an outer layer on top of the cell wall which operates as a barrier to antibiotics (India, 2015). It also has enzyme(s) which convert active drugs to inactive form (Abiba, 2013). The Gram-positive bacteria are prone to herbal extracts which is explained by the bacteria cell wall composition, which is a polymer permeable to many substances (India, 2015). This is supported by Abiba (2013) whose work indicated that *B. subtilis* was more susceptible whereas *S. typhi* was resistant to herbal drugs. Antimicrobial compounds have been successfully isolated from various medicinal plants according to ethnobotany survey (India, 2015).

2.10 Plants under the study

2.10.1 *Zanthoxylum chalybeum*

Plate 2.1: Plant specimen of *Z. chalybeum* taken in January, 2018; Meru County

Zanthoxylum chalybeum (Engl.) *Var. chalybeum*, belong to Rutaceae family (Olila, 2001). This plant is found in dry areas of Tigania West, Meru County. *Z. chalybeum* is known as 'Muluiri' by Tigania community. The plant grows up to a height of 12 m with its drooping branches growing and spreading up to 1.5 m forming spreading canopy (India). The stem back of *Z. chalybeum* plant is dark and prickly on outer surface. The branches have scattered thorns. It is a woody plant with relatively broad, pointed apex, bi-pinnate shiny leaves with smooth margin and white terminal peduncle flowers (Olila, 2001). This plant has been used by local community to treat coughs, pneumonia and gastro-intestinal disorders. Many more uses include firewood, charcoal and housebuilding materials (Olila, 2001). It occurs on rocky ground in close association with other thickets of plant species and is widely distributed in dry locality of Tigania Sub County, Meru County.

2.10.2 *Aloe deserti*

Plate 2.2: Plant specimen of *A. deserti* taken in January, 2018; Meru County

Aloe deserti (A. Berger), belong to Liliaceae family (Renisheya, 2012). This plant is found in Buuri, Meru County. *Aloe deserti* is known as 'Chukurai' by Imenti community. *Aloe deserti* is a stemless perennial drought resistant plant growing on sandy ground, approximately 100 cm tall, spreading offsets with white-yellow terminal flowers (Udgire, 2014). It has thick pointed green leaves with thorny margin and contains (mucilaginous jelly) water supply for the plant to survive drought periods (Udgire, 2014). This plant has been used extensively by local community to treat malaria, wound healing, stomach ailments and gastro-intestinal disorders (Renisheya, 2012). Many more uses include bee forage and livestock herbal drugs. It occurs in clusters, in close association with other thickets of plant species and is sparsely distributed in dry locality of Buuri Sub-County, Meru County.

2.10.3 *Zanthoxylum usambarense*

Plate 2.3: Plant specimen of *Z. usambarense* taken in January, 2018; Meru County

Zanthoxylum usambarense (Engl.) Kokwaro, belong to Rutaceae family (Kokwaro, 2009). This plant is found in Igembe North, Meru County. The plant is known as 'Mubuchwa' by Igembe community. The plant grows up to a height of 14 m high often multi-stemmed with its branches growing and spreading up to 2 m forming conical canopy bearing outgrowths (Musyoka, 2016). The stem back of *Z. usambarense* plant is greyish with dark straight thorns on outer surface (Olila, 2001). It is a woody plant with relatively broad, bi-pinnate deep green leaves with smooth margin and creamy-white terminal flowers (Musyoka, 2016). This plant has been used by local community to treat coughs, tooth ache, rheumatism and gastro-intestinal disorders (Abiba, 2013). Many more uses include firewood, charcoal and housebuilding materials. It occurs individually, in close association with other thickets of plant species and is widely distributed in Nyambene hills of Meru County.

2.11 Biochemical markers

2.11.1 Alanine aminotransferase (ALT)

Alanine aminotransferase is found in cytoplasm of body cells and drains to plasma in various body tissues, especially in the liver (Rahul, 2005). ALT biomarker indicates cytoplasmic and mitochondrial hepatocellular injury. Though damages to other body organs increase ALT levels, ALT is increased with liver damage and is used to monitor liver injuries and/or screen liver disease (Abiba, 2013).

2.11.2 Aspartate aminotransferase (AST)

Aspartate aminotransferase is found in cytoplasm and mitochondria of body cells of organs such as heart, kidney, liver, pancreas, muscles, brain and lungs (Murugi, 2012). In healthy persons, levels of AST in the blood are low. Upon body tissues such as heart and liver damage, the enzyme is released into blood serum (liquid part of blood) (Gaelle, 2020). When injury is mild, AST comes from the cell cytoplasm. Severe tissue injury results in more of mitochondrial enzyme being released to blood. High levels of AST can be found in cases such as liver damage, viral hepatitis and carbon tetrachloride poisoning (Arika, 2016). Moderate production is seen in muscle injury. When either liver or muscles are damaged, they release AST into the blood stream, but this depends on period taken before the blood is investigated following injury. Thus, AST serum increases after eight hours and reaches maximum within 24-36 hours and get restored in seven days (Abiba, 2013). This AST maker indicates liver damage and is also used to monitor disorders such as pancreatitis (Gaelle, 2020).

2.11.3 Alkaline phosphatase (ALP)

Alkaline phosphatase is essential in biochemical reactions in the human body. It is mostly contained in the bones, kidney, liver and digestive system. It is also a diagnostic tool of liver or bone disease (Arika, 2016). Alkaline phosphatase is taken to the liver by bile tubes. Once the liver is damaged, blockage of bile tubes or ducts occurs (cholestatic) or has a liver disease (hepatocytosis) and ALP gets out of the liver into the blood stream (Gaelle, 2020). High levels of ALP indicate liver disease and also bone disorders. Its concentration in blood stream is used as biomarker in helping to determine diagnoses such as hepatitis or osteomalacia. The kidney tumors and infections also raises the level of alkaline phosphatase in blood stream. High alkaline phosphatase levels in liver indicate blocked bile duct and mononucleosis (liver swelling) (Abiba, 2013). Low levels of alkaline phosphatase indicate hypophosphatasia, a genetic disorder that affects bones. This diagnostic tool is very important in differentiating the type of liver disease, especially cholestatic and hepatocytosis (Murugi, 2012).

2.11.4 Gamma-glutamyl transpeptidase (GGT)

Gamma-glutamyl transpeptidase is found in the cell membranes of organs, such spleen, heart, brain, kidneys and pancreas. GGT helps in the transfer of amino acids across the cellular membrane (Gitimu, 2015). High levels of serum GGT activity are found in diseases of the liver and pancreas. GGT in atherosclerotic plaques suggest a potential role in pathogenesis of cardiovascular diseases (Gitimu, 2015). GGT circulates in blood in the form protein aggregates which indicate metabolic syndrome and chronic liver disease (Kamisha, 2006). The enzyme GGT is also elevated by

heavy intake of alcohol. The disproportionate elevation compared to other liver enzymes (such as ALP or ALT) indicate alcohol abuse (Kamisha, 2006).

2.11.5 Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent and it performs the reverse reaction during the Cori cycle in the liver (Gitimu, 2015). Lactate dehydrogenase exhibits feedback inhibition, such that pyruvate conversion to lactate is decreased. The enzyme is released after tissue breakdown indicating hemolysis. Other disorders indicated by elevated LDH include meningitis, encephalitis, acute pancreatitis, and HIV (Joseph, 2002).

2.11.6 Alpha-amylase (AMYL)

Alpha-amylase is used in the diagnosis of pancreatitis. Amylase is the main hematological chemical marker of pancreatitis in patients (Gitimu, 2015). Other conditions such as non-malignant hepatobiliary, gastrointestinal diseases, sepsis, pulmonary failure and subdural bleeding increase pancreatitis (Fody, 2010).

2.11.7 Bilirubin (BIL)

Bilirubin occurs in the catabolic pathways in the body for the clearance of the waste products. There two types of bilirubin that is unconjugated/indirect and conjugated/direct bilirubin. Total bilirubin (TB) refers to both unconjugated and conjugated bilirubin (Saha, 2017). Bile ducts injury elevates amout of direct and indirect bilirubin in blood (Gitimu, 2015). Unconjugated (indirect) bilirubin (I-BIL) is

bound to albumin and this facilitates its transport to the liver (Soetan, 2010). In the liver cells, glucuronic acid is added to unconjugated bilirubin (not water soluble) by the enzyme glucuronosyltransferase. These forms conjugated bilirubin (D-BIL), which is water soluble and excreted through bile duct and through kidney (Gitimu, 2015). Increase in conjugated bilirubin in blood indicates a liver disease. More than 50 % increase of conjugated bilirubin indicates cholestasis (Fody, 2005).

2.11.8 Creatinine kinase (CK)

Creatinine kinase is an enzyme that is found in the body muscles. It is a catalyst in muscle that speeds up energy (ATP) conversion process (Arika, 2016). Of 95% CK originate from the skeletal muscles, as a result of drainage from body muscles during the strenuous activities (Gaelle, 2020). The physically fit and active individuals indicate high CK activity which depends on age, gender and race or ethnicity. The absolute CK activity lies within 22-198 U/L of serum in individuals (Aniagu, 2004). The normal CK levels in man ranges from 38-174 U/L, while a woman's CK levels range from 96-140 U/L. This is because man's body has more muscle mass than that of a woman. It is used to detect swelling of muscles (myositis) or muscle damage (myopathies) (Gaelle, 2020). It also used to diagnose rhabdomyolysis, which is a severe breakdown of muscles in humans (Aniagu, 2004).

2.11.9 Glucose (GLU)

Glucose can be too much (hyperglycemia) or too low (hypoglycemia) in the human body. Knowing glucose levels in body can be used in the diagnosis and treatment of carbohydrate metabolism disorders (Soetan, 2010). The presence of glucose in urine is common in healthy, pregnant women. Glucose is not present in urine of a person

with no body physiological function. Examination of cerebrospinal fluid (CSF) glucose assist in distinguishing bacterial menengitis and viral meningitis. Glucose level is usually low (less than 40% to 45%) in bacterial meningitis and tuberculous meningitis patients. Carcinomatous meningitis exgerates CSF glucose level below the normal range (Gitimu, 2015).

2.11.10 Cholesterols (C)

Cholesterols are used to diagnosis lipid and lipoprotein metabolism disorders. Total cholesterol (TC) is examined and its values are used for treatment of atherosclerosis, hyperlipoproteinemia and thyroid diseases (Noh, 2022). High density lipoproteins (HDLs) are lipid protein complexes that demonstrate the strong and independent inverse association between HDL-C and the risk of coronary artery disease (Noh, 2022). The transports of HDL-C to liver from other tissues protect the body against atheromatous plaques. LDL-C indicates clogging of arteries (arteriosclerosis) causing chest pain called angina (Soetan, 2010).

2.11.11 Triglycerides (TG)

Triglycerides play an important role in metabolism and are excellent energy storage molecules (Soetan, 2010). Triglycerides are examined and results used for treatment in patients with liver obstruction, diabetes mellitus, endocrine disorders, lipid metabolism disorders. Triglyceride analysis is used to help classify the various metabolic lipoprotein disorders. Triglyceride helps in assessment of atherosclerosis, a coronary artery disease (Soetan, 2010).

2.11.12 Creatinine (CREAT)

Creatinine is synthesized in the liver from the methylation of glycocyamine and transported through blood to muscles (Soetan, 2010). Creatinine is filtered out of the blood by the kidneys. In case of faulty filtering or kidney failure, creatinine blood levels rise due to poor clearance by kidneys. Creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which indicate glomerular filtration rate (GFR). The GFR is clinically used to measure renal functioning (Kaplan, 2003). Elevated blood creatinine level is observed in damaged nephrons, this makes it unsuitable for detecting early-stage kidney disease (Frank, 2005).

2.11.13 Uric acid (UA)

Uric acid is a product of the metabolic breakdown of purine nucleotides. High uric acid in blood leads to gout which causes pain in joints and capillaries resulting from needle-like crystals of uric acid. Uric acid is also associated with diabetes and kidney stones (Soetan, 2010). Xanthine oxidase makes uric acid from xanthine and hypoxanthine. Uric acid can be increased as result of hereditary, reduced excretion and rapid weight loss (Weaver, 2008). Kidney stones can also be formed by deposition of sodium urate microcrystals (Schlesinger, 2010).

2.12 Hematological markers

2.12.1 Erythrocytes

Erythrocytes are enucleated blood cells that contain red respiratory pigment called hemoglobin. Hemoglobin gives blood its red color (Thrall, 2002). Hemoglobin carries oxygen in form of unstable compound known as oxy-hemoglobin (Arika, 2016) to tissues for breakdown of glucose releasing energy as per the body demand. There are

about 3.9-5.9 million/μl red corpuscles in women and 4-6 million/μl red corpuscles in men (Howard, 2002). The low levels of red blood cells in blood are linked with anemia condition (Gaelle, 2020). Erythrocytosis is induced by tissue hypoxia. This occurs due to increased production of erythropoietin either as a consequence of hypoxemia or because of kidney damage (Haslett, 2002).

2.12.1.1 Hemoglobin

Hemoglobin (Hb) is an iron-rich protein contained in red blood cells (Thrall, 2002). Oxygen diffusing into the lungs combines with hemoglobin to form unstable compound oxyhemoglobin which carries it to tissues (Arika, 2016). Hemoglobin is expressed in g/dL of blood (Barrell, 2017). Low levels of Hb leads to low levels of oxygen and body is left short of the oxygen. This condition is called anemia. Anemia can be as a result of iron deficiency or destruction of red blood cells (Abiba, 2013). The synthesis of hemoglobin from abnormal gene results in sickle cell anemia, a disease characterized by sickle shaped erythrocytes and so carries less oxygen. This leads to insufficient supply of oxygen to body tissues. High hemoglobin levels indicate a blood disease, referred to as polycythemia (Barrell, 2017).

2.12.1.2 Packed red cell volume

Packed red cell volume (PCV) is the oxygen carrying capacity. The PCV levels can be tool low or tool high and this indicate a blood disorder, dehydration, over-hydration or other medical conditions. Low PCV suggests anemia that is a decrease in the total amount of red blood. The oxygen carrying capacity is not enough to meet the body's physiological needs. High values of PCV can be due to polycythemia, erythrocytosis or hemochromatosis (Chidozie, 2020).

2.12.1.3 Mean corpuscular hemoglobin

Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin found in a blood sample. MCH values vary inversely to MCV values. If the size of red blood cell is large (as assessed by MCV), the amount of hemoglobin per red blood cell is high (as assessed by MCH) and vice versa (Chidozie, 2020).

2.12.1.4 Mean corpuscular hemoglobin concentration

Mean corpuscular hemoglobin concentration (MCHC) is the concentration of hemoglobin in red blood cells (Glan, 2014). MCHC indicates whether the person's blood red cells have more or less compound that carries oxygen. Low MCHC indicates hypochromic or anemic condition. High MCHC indicate hyperchromic or hemolytic condition where white blood cells attack red blood cells (Chidozie, 2020).

2.12.1.5 Mean corpuscular volume

Mean corpuscular volume (MCV) gives the actual size of red blood cells. Low MCV indicates red blood cells does not have adequate haemoglobin (microcytic) (Glan, 2014). High MCV indicate red blood cells are large in size leading to melagocytosis (Chidozie, 2020).

2.12.1.6 Red cell distribution width

Red cell distribution width (RDW) is the degree of variation of the size of red blood cells (Theodoras, 2020). This gives complete blood count and describes variation in the size of circulating erythrocytes. It helps to diagnose various types of anemia such as iron deficiency and sickle cell disease. Elevated RDW indicate presence of coronary artery disease (Glan, 2014).

2.12.2 Leucocytes

Leucocytes have no pigment and appear colorless (Thrall, 2002). They provide immunity to the body against infections or foreign materials. There are about 4,500 to 11000 white blood cells per mm^3 of blood in a health adult human (Arika, 2016). Leucocytes squeeze (using amoeboid motion) through openings (pores) in blood vessels by a process called diapedesis. They kill bacteria by phagocytosis (Arika, 2016). There are two groups of white blood cells, classified based on cytoplasm (granulocytes and agranulocytes) (Thrall, 2002). Eosinophils, basophils and neutrophils are called granulocytes. Monocytes and lymphocytes are known as agranulocytes (Thrall, 2002). The increase in WBCs is known as leukocytosis, whereas a decrease is known as leukopenia (Abiba, 2013).

2.12.2.1 Neutrophils

Neutrophils (NEU) are the most numerous and body's main defenses against infections (Thrall, 2002). They are multi-lobed, short-lived cells and consist of 60-70% of circulating leucocytes in humans (Horward, 2002). Neutrophils kill disease causing micro-organism by ingestion, a process called phagocytosis. Increase in neutrophils is due to organ damages and infections. The low levels (neutropenia) of neutrophils can occur as a result of HIV/AIDS infections (Horward, 2002).

2.12.2.2 Lymphocytes

Lymphocytes (LYM) are grouped into two categories, B-lymphocytes and T-lymphocytes. Their main function is recognition of foreign agents in the body. B-lymphocytes release antibodies which are proteins that clamp and destroy specific

micro-organisms in body tissues (Thrall, 2002). T-lymphocytes recognize malignant cells and kill them or assist B-cells to synthesis antibodies (Horward, 2002).

2.12.2.3 Monocytes

Monocytes (MON) are agronulocytes which enter connective tissues and differentiate into macrophages (Thrall, 2002). They combine with lymphocytes and help in recognizing immunocompetent cells (Arika, 2016). They have a longer lifespan of 12 to 100 hours. These cells also destroy damaged or dead cells in the body. Body inflammation results into monocytosis (Howard, 2002).

2.12.2.4 Basophils

Basophils (BAS) are phagocytic cells that circulate in the blood of humans and other animals. Basophils are characterized by distinctive morphologic features such as irregular surface, multi-lobed nuclei and dense cytoplasmic granules. They have anti-tumorigenic properties through release of pro-inflammatory mediators TNF-α and tryptase (Chanhan, 2022).

2.12.2.5 Eosinophils

Eosinophils (EOS) are granulocytes that develop during hematopoiesis and contribute to immune system, helping to fight diseases and infections. They are also responsible for the mechanisms associated with allergic responses in the body. The rapid increase in the number of eosinophils indicates an invasion by pathogens (Davis, 2017).

2.12.3 Platelets

Platelets (PLT) also called thrombocytes are fragments of mature megakaryocytes. Pseudopod of megakaryocytes breaks and forms platelets through the process of cytokinesis. Platelets are disc-shaped and contain lysosomes. They help in hemostasis and angiogenesis. In a healthy person, platelet ranges between 150000 and 450000 in each microliter of blood. High platelet levels indicate thrombocytosis. Low levels indicate thrombocytopenia (Haoqi, 2020).

2.12.3.1 Platelet distribution width

Platelet distribution width (PDW) reflects how uniform platelets are in size (Saffaa, 2020). The average size of platelet ranges between 8.1fL and 25.0 fL. High PDW indicate large platelet size variation which may result in cardiovascular disorders, thyroid malignancies and certain cancer. Low PDW indicate anemia (excessive bleeding) (Karolina 2020).

2.12.3.2 Mean platelets volume

Mean platelet volume (MPV) is the index that expresses average size of platelet in blood (Saffaa, 2020). In a healthy person, it ranges between 7.2 and 11.7 fL (Karolina, 2020). Elevated MPV indicate platelet hyperdestruction. Decreased MPV indicate platelet hypodestruction (Aleksandra, 2019).

2.13 Active chemicals in medicinal plants

Plants contain organic compounds that are used in management of human diseases (Koche, 2010). These organic compounds act as defense mechanisms against pathogenic attack in plants (Sule, 2011). Active chemicals accumulate in various plant

organs such as stem, roots and leaves (Rao, 2003). Many studies have shown that plants contain phytochemicals which have`physiological action on the human body (Khan, 2011; Manta, 2013). These phytochemicals are of great importance to the health of individuals and communities (Sule, 2011).

2.13.1 Alkaloids

Alkaloids are the largest group of phytochemicals synthesized by medicinal plants (Abiba, 2013). These compounds are mainly involved in the plant defense against herbivores and pathogens (India, 2005). Examples of alkaloids that are used in clinical settings include morphine, quinine, strychnine, ephedrine and nicotine. Studies have reported that alkaloids have antimicrobial activities (India, 2005).

2.13.2 Flavonoids

Flavonoids are polyphenolic structures found in roots and stems (Bii, 2010). They are highly concentrated in outer most layers of plant parts (Orhan, 2010). Various studies have reported that flavonoids enhance human immunity (Nyamani, 2016). They also exhibit powerful biological activities such as antiviral, antibacterial and insecticidal (Fukai, 2002). These compounds interfere with nucleic acid of microorganisms (Panche, 2016). Flavonoids have provided valuable medicines for sickness in humans and animals (Panche, 2016).

2.13.3 Saponin

Saponins are glycosides found in large amount in plants. Saponins serve as lytic agents because they have detergent properties (Soetan, 2006). They have been used economically in various areas such as drugs, cosmetics and detergents. (Eskander,

2006). Various studies have reported that saponins have antimicrobial activities such as antifungal antibiotic and antiviral (Soetan, 2006).

2.13.4 Tannins

Tannins are high molecular weight substances that form reversible and irreversible compounds polysaccharides (Nyamani, 2016). Tannins accumulates more in plant parts such as leaves and flowers (Frutos, 2004). These compounds are increased by high temperatures and extreme light intensities (Frutos, 2004). Various studies have reported that tannins prevent development of microorganisms by precipitating microbial proteins or inactivating cellular enzymes (Abiba, 2013).

2.13.5 Terpenoids

Terpenoids are synthesized by dimethyl diphosphate and isopentenyl diphosphate compounds in plants (India, 2005). Examples of common terpenoids are menthol, camphor (monoterpenes) and sesquiterpenoids. Terpenoids are active against bacteria, but their mechanism of killing bacteria is unknown (Abiba, 2013). It is assumed that terpenoids destroy cell wall of bacteria (Nyamani, 2016).

2.13.6 Anthraquinones

Anthraquinones are phenolic compounds, biosynthesized from tricyclic aromatic compound in plants (Gaspar, 2018). Various studies have reported that anthraquinones have antibacterial properties (Odongo, 2013). Anthraquinones also exhibit powerful antiviral, anti-inflammatory and anti-cancer agents (Gaspar, 2018) resulting from hydroxyls at the C-1 and C-8 of the anthraquinone (Gaspar, 2018).

2.13.7 Glycosides

Glycosides are organic compound of plant origin and comprises of sugar linked to non-sugar moiety or glycone (Bernel, 2011). Glycosides are classified based on the glycone component. Steroidal glycosides are of pharmacological importance. Flavonoid glycosides exhibit chemical defense system in plants and in plant-insect interactions. Glycosides made of monosaccharides have antioxidant, antihypertensive and antibacterial activities (Bernel, 2011).

2.13.8 Reducing sugars

Reducing sugars are organic compounds that possess terminal aldehyde group or a ketone group (Deepa, 2020). The main reducing sugars in plants is both as nutrients and as regulatory molecules that modulate metabolism, stress response, disease resistance and gene expression related to growth of plant. These sugars control metabolic pathways and help in the production of secondary metabolites that enhance antibacterial and antioxidant activities in plants (Deepa, 2020).

2.14 Review of mineral elements

Minerals are inorganic substances found in the tissues and fluids of human body. They are in two categories: macronutrients and micronutrients. Calcium (Ca), Magnesium (Mg) and Potassium (K) are considered as macronutrients because they are required in amount greater than 100 mg/dL. Micronutrients such as Manganese (Mn), Zinc (Zn), Iron (Fe), Lead (Pb), Chromium (Cr), Copper (Cu), Arsenic (As), Vanadium (V), Strontium (Sr) and Mercury (Hg) are required in amount less than 100 mg/dl (Murray, 2000). These minerals should be consumed according to the needs of organisms (Siddiqui, 2014). Excess intake interferes with the normal functioning of

the body. Mineral elements responsible for antimicrobial activity and their toxicity are described below:

i) Potassium (K): Potassium helps in prevention of osteoporosis, atherosclerosis and heart diseases. It is also involved in acid-base balance, protein synthesis and activator of enzymes. Potassium metal has antibacterial activity against *S. aureus* and *E. coli* (Sutrisno, 2020). Diseases caused by elevated potassium levels include cardiac arrest and bowel ulcers. Depletion of potassium in body results to reduced glucose tolerance and diabetic acidosis (Soetan, 2010).

ii) Magnesium (Mg): Magnesium is an active component of enzyme systems in which thymine phosphate is a cofactor (Soetan, 2010). It also supports the immune system (Shivani, 2012). Magnesium has antibacterial properties against Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria (Nguyen, 2018). Low levels of Mg can increase risk of cardiovascular problems and osteoporosis. Mg overdose can cause muscle paralysis (Lusine (2019).

iii) Calcium (Ca): Calcium is a regulator of biological functions such as blood clotting, neuro-information transmission and heart rhythms (Soetan, 2010). Calcium exhibits antimicrobial activity. Dissociation of $Ca(OH)_2$ releases calcium ions (Ca^{2+}) in aqueous suspension that contributes to antimicrobial activity against Enterococcus faecalis (Mohammadi, 2012). Overdose of calcium can cause extreme tiredness and abnormal heart rhythms (Lusine (2019).

iv) Copper (Cu): Copper is involved in biological processes (Shivani, 2012). It is also considered as anti-cancer, anti-inflammatory and synergistic agent. It is able to kill multi-drug resistant bacteria together with antibiotic (Sirisha, 2018). Cupper complexes have anti-microbial activity against *B. cereus* and *E. coli* (Carla, 2019). Copper overdose interferes with bone metabolism and reduce bone cortex strength. Other symptoms include nausea and diarrhoea which are due to the irritant effect of copper on gastrointestinal mucosa (Carla, 2019).

v) Zinc (Zn): Zinc is required for catalytic activity of various enzymes, immune systems and DNA synthesis (Soetan, 2010). Dissolution of zinc oxide particles releases zin ions (Zn^{2+}) in aqueous suspension that contributes to antimicrobial activity (Shivani, 2012). Antibacterial activity of zinc ions against *Streptococci* and *Staphylococcus* depends on its ability to bind cell membrane thereby prolonging the lag phase of cell cycle. *Streptococci* and *Staphylococcus* takes more time to complete cell division hence dying (Shivani, 2012). However excess intake may lead to symptoms such as nausea and tiresome (Carla, 2019).

vi) Manganese (Mn): Manganese acts as an activator for many enzymes (Shivani, 2012). It also involved in amino acid / protein and carbohydrate metabolisms. Manganese complexes have anti-inflammatory activities and those containing 1, 10-phenanthroline and dicarboxylate ligands inhibit viability of *Mycobacterium tuberculosis* (Andris, 2021). Overexposure of manganese can cause CNS injury, oxidative stress and apoptosis dystonia (Tan, 2006). Diseases associated with manganese toxicity include psychotic symptoms and Parkinsonism (Carla, 2019).

vii) Vanadium (V): Vanadium plays an important role in metabolic processes (Rafiq, 2022). Vanadium ligands have potential antibacterial activity (Kasimbi, 2019). Gastrointestinal discomfort can occur after administering vanadium salts to a sick person. The organically chelated compounds cause mild intestinal irritation than vanadium salts (Soetan, 2010).

viii) Iron (Fe): Iron is essential for biochemical activities, such as oxygen transport, synthesis of deoxyribonucleic acid (DNA) and electron transport (Mathieu, 2009). It is also a component of cytochromes (Soetan, 2010). Iron free radicals /hydroxyl radicles kills both Gram negative (*E. coli*) and Gram positive (*S. aureus*) bacteria (Carla, 2019). Iron overdose results in progressive lesions in the pancreas, liver and heat. Impaired glucose metabolism indicates iron overload (Soetan, 2010).

ix) Chromium (Cr): Chromium is an element that influence metabolism of lipid, carbohydrate and protein (Uppala, 2005). Chromium has also been identified as the active ingredient of the glucose tolerant factor (Brown, 2003). Chromium deficiency leads to development of atherosclerosis. Chromium poisoning in humans is usually limited to accidental ingestion of chromic acid or chromates (Mahdi, 2021).

x) Mercury (Hg): Mercury is used in redox reactions (Sirisha, 2018) and catalytic activity (Soetan, 2010). Mercury is used as antimicrobial agent. Dental amalgam used in tooth fillings has bacteriostatic activity (Sirisha, 2018). However excess intake of mercury leads to CNS injury and hepatotoxicity (Mahdi, 2021).

xi) Strontium (Sr): Strontium is the most hazardous radioactive and has direct impact on bone metabolism (Soetan, 2010). Strontium ions in the network of bioactive glasses induce antimicrobial activity against *Porphyromonas gingivalis*. The effectiveness is a direct relationship with concentration of Strontium in the glasses (Sara, 2020). Radioactive strontium is absorbed and deposited in tissues especially the bones, and is also readily transmitted to the foetus (Soetan, 2010).

xii) Arsenic (As): Arsenic helps in physiological functions, such as methionine metabolism during pregnancy (Nandi, 2006). Arsenic action is similar to that of antibiotics and appears to control harmful intestinal microorganisms (Sirisha, 2018). Exposure to high amount of arsenic leads to malignancies, gastrointestinal toxicities, cardiac arrhythmias and cardiovascular diseases (Soetan, 2010).

xiii) Lead (Pb): Lead has no known biological function in body. Lead metal has antibacterial activity agaist *Escherichia coli* and *Staphylococcus aureus* in hospitals and food processing biofilms (Miyano, 2010). Chronic lead poisoning is commonly seen in young children arising from sucking lead paint or lead toys and is also seen in workers engaged in printing, paint and petroleum industries (Mahdi, 2021). Reproductive dysfunction by lead has distinct morphological and biochemical features including decreased sperm quality and altered sperm morphology and low androgen levels (Soetan, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling sites

The study area was Meru County (Figure 3.1), located at latitude 0° 20' North and 0° 10' South, and longitude 38° 25' East of Greenwich. Meru County borders Tharaka-Nithi County to South West, Isiolo County to North, Nyeri County to South West and Laikipia County to West. The County is made up of nine administrative Sub-Counties. The County has a cosmopolitan population of 1,545,714 and an area of about 7,000.3 km^2 (KNBS, 2019). Meru County is enriched with many essential resources. Annual rain falls twice. The highest rainfall (2600 mm) falls between November and January and lowest rainfall (1800mm) falls between March and June with dry spells from August through September to October. The altitude varies from 1450 m on dry part to 2700 m in highlands (foothill of Mount Kenya and Nyambene hills) (KIG, 2015).

The county's economy mostly relies on agriculture; that is subsistence (growing food crops such as beans, maize) and commercial farming (growing cash crops such as tea, coffee, miraa/khat) (KIG, 2015). The vegetation in the county includes forests in highlands and bushlands, grasslands and shrubs in lowlands of Tigania West, Tigania East, Igembe North and Buuri Sub-Counties bordering Isiolo County. These areas are only suitable for livestock keeping and game reserves. The county is the home of Meru National Park, the habitat to a variety of wildlife including Gazelles, Elephants, Hyenas, Giraffe, Zebra, Buffalos and many more making the park a major tourist destination (KIG, 2015).

Meru County

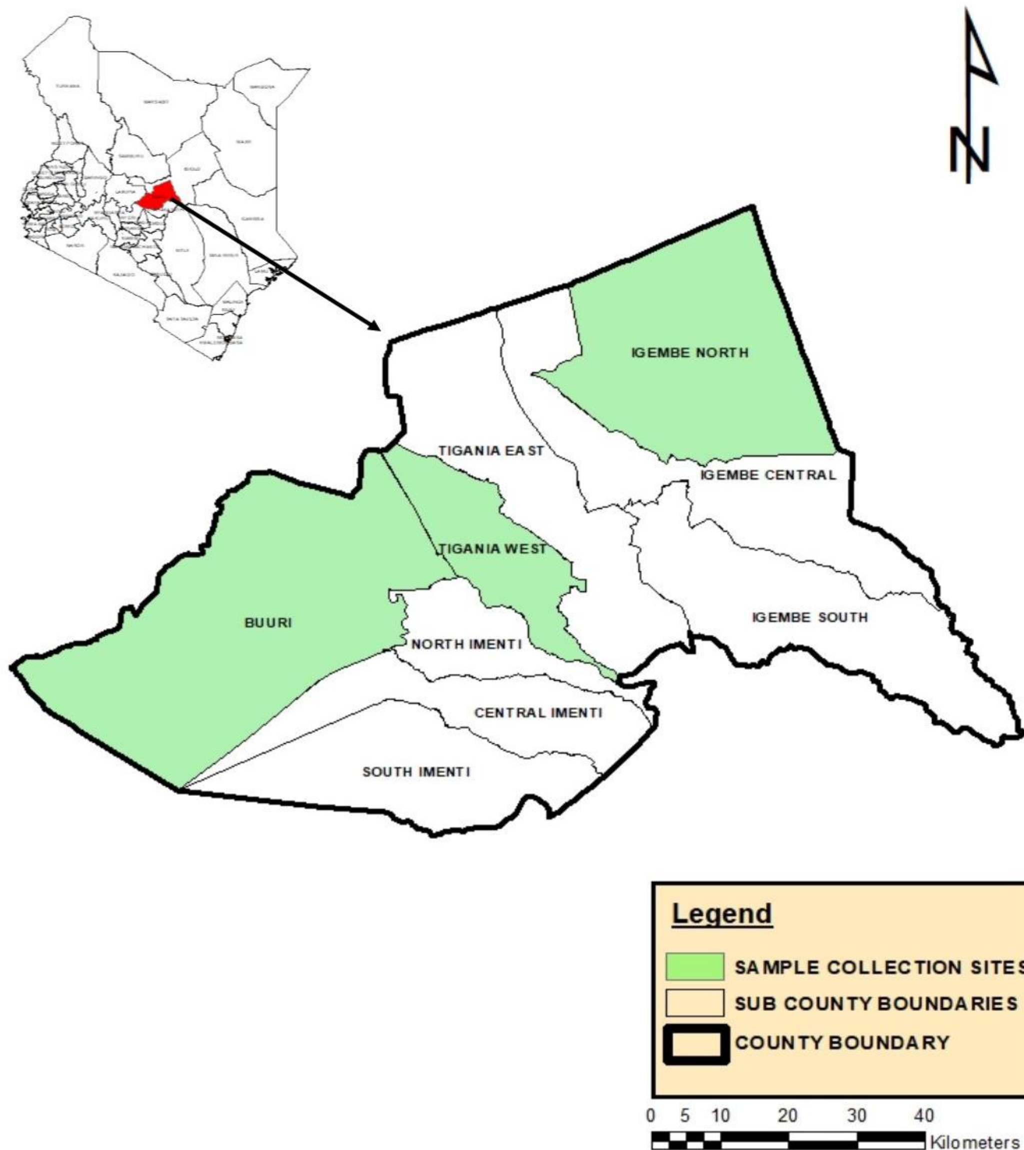


Figure 3.1: The map of Meru County showing Sub-Counties where medicinal plants under study were sampled.

3.2 Plant parts used for medicinal purposes

The phytochemicals are found from various part of a plant such as leaves, roots, backs, seeds and flowers (Odongo, 2013). Active ingredients are in higher concentrations in leaves, roots and barks (Neli, 2012). Roots and stems have exudates which act as toxin for pathogenic attack and these exudates are of medicinal value to human body (Gidey, 2010). The plant leaves can be used singly or can be mixed with

twigs (Neli, 2012). The presence of bioactive compounds in plant are influenced by several factors such as environmental changes, plant part used and plant age (Odongo, 2013). Old plants have high concentration of phytochemicals compared to young plants (Okoli, 2007).

3.3 Collection of plant materials

The plant samples were collected from Buuri, Tigania West and Igembe North Sub-Counties of Meru County. The samples comprised of barks and leaves obtained by cutting them using a sharp panga (Plate 3.1).

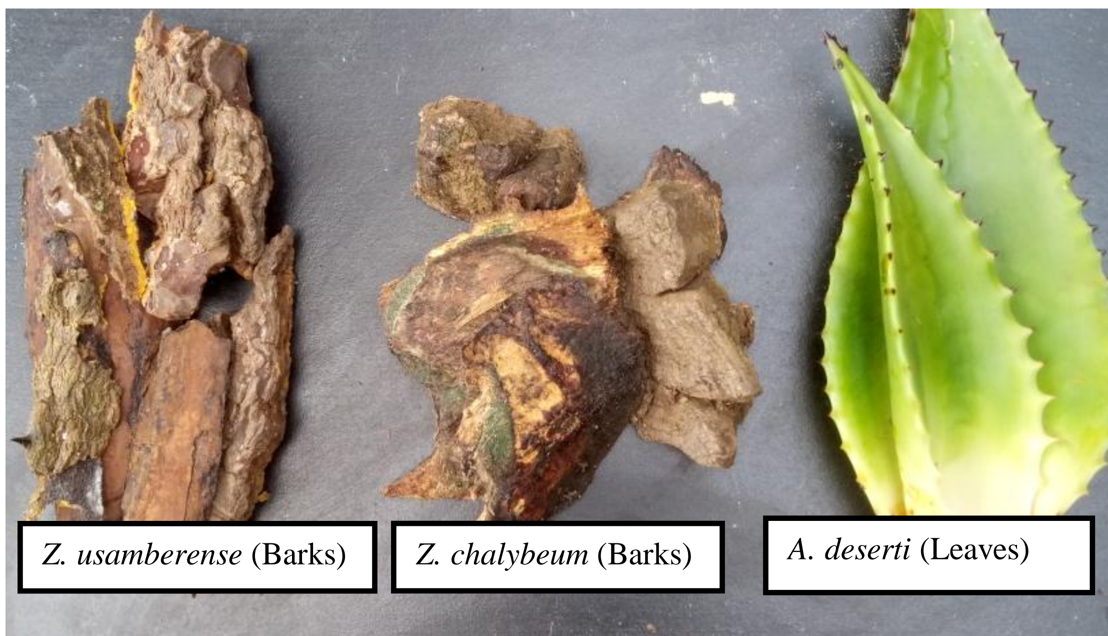


Plate 3.1: Plant parts used in the study

The material of each plant was collected randomly and in triplicate. They were then mixed thoroughly and carried to laboratory. The plant twigs/leaves were autheticated at the East African Herbarium, Kenya. The voucher numbers SM01 (*A. deserti*), SM02 (*Z. chalybeum*) and SM03 (*Zanthoxylum usambarense*) (Appendix 5) were deposited at Herbarium of Plant and Microbial Sciences Department, Kenyatta

University, Kenya. The coordinates taken using a GPS machine (Garmin extrex H) in Table 3.1 indicate sites of the collected plant materials.

Table 3.1: Coordinates of site of collection of plant materials

Plant species	Latitude	Longitude	Altitude
Zanthoxylum usambarense	0.331067°N	37.936792°E	1828.5M
Aloe deserti	0.221158°N	37.621957°E	1479.0M
Zanthoxylum chalybeum	0.021152°N	37.636718°E	1459.0M

3.4 Preparation of plant materials

The fresh materials were cleaned carefully to remove foreign materials and spread to dry at room temperature for 14 days. The chopped materials were grounded into coarse powder using Warring Blender (for leaves) and Wily Mill (Mode No. 2, USA) (for stem barks) in the Animal Breeding Laboratory, Kenyatta University. The powders were sealed in polythene bags and kept in lockable cupboards waiting for further analysis (Midiwo, 2010).

3.5 Extraction of plant materials

One hundred and fifty grams (150g) of powdered material of each plant was transferred into a conical flask with 1000 ml of methanol and left overnight (Kitonde, 2013). The supernatant was filtered using Whatman No. 1 filter paper into clean conical flask (Midiwo, 2010).

3.6 Concentration of extract

The rotary evaporator (Bibby RE 100) was used to evaporate methanol and obtain crude extract at a temperature of 40^0C (Omwenga, 2009). The paste was collected using a vial which was put in evacuated desiccator with anhydrous copper sulphate to dry the extract. The extract was weighed and stored in the refrigerator at -20^oC (Omwenga, 2009).

3.7 Qualitative phytochemicals analysis

The qualitative analysis of phytochemicals was performed to establish the presence of secondary metabolites such as alkaloids, saponins, flavonoids, tannins, terpenoids anthraquinones, reducing sugars and glycosides in methanol extract using standard qualitative assays (Teshager, 2016).

3.7.1 Alkaloids test

One and half milliliter of 1 % HCl acid was added to 0.5 g of plant extract. The formation of a reddish-brown color with draggendroff's reagents (potassium bismuth iodide solution) was regarded as positive presence of alkaloids (Farhat, 2011).

3.7.2 Tannins test

One and half milliliter of 0.1 % of $FeCl_3$ was added to 0.5 g of plant extract. A brownish green color indicated positive presence of tannins (Bhawana, 2015).

3.7.3 Flavonoids test

One and half milliliter of 2 % solution of NaOH was added to 0.5 g of plant extract. The turning of the yellow color to colorless on addition of two drops of 1 % HCl acid dropwise indicated presence of flavonoids (Bensode, 2012).

3.7.4 Saponins test

Five milliliter of distilled water was added to 0.5 g of methanol plant extract. Three drops of olive oil (shaking vigorously) and emulsion formed indicated presence of saponins (Farhat, 2011).

3.7.5 Glycosides test

Half a gram of plant extract was dissolved in 2 ml of chloroform. Two milliliters of concentrated H_2SO_4 acid were added carefully to the mixture (shaking). The presence of a steroidal ring (reddish brown) indicated presence glycosides (Njoku, 2009).

3.7.6 Anthraquinones test

Three milliliter of benzene was added to 0.5 g of plant extracts and filtered. Five milliliters of 10 % ammonia were added to the filtrate (shaking) and observed for indication of pink, red or violet in the ammonical (lower) phase indicating the presence of anthraquinones (Njoku, 2009).

3.7.7 Terpenoids test

Two milliliter of chloroform was added to 0.5 g of methanol extracts. Three milliliters of concentrated H_2SO_4 were added (carefully) and the reddish brown formed indicated the presence of terpenoids (Farhat, 2011).

3.7.8 Reducing sugar test

Two milliliter of water was added to 0.1g of plant extract. Two milliter of Felling's reagent was added and the content put in water bath for 3 minutes. The Orange or brown colour indicated the presence of reducing sugar.

3.8 Determination of metal levels in herbal extracts

3.8.1 Instruments

Analysis of heavy and trace metals in the extracts were assessed using atomic absorption spectrophotometer (AAS-Model: 210 VGP). The flame absorption mode

was used with air-acetylene and nitrous oxide acetylene flame (Ram, 2011). The operating conditions of AAS are given in Appendix 3.

3.8.2 Chemicals and Glass wares

Nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were used for digestion of herbal extracts (Sahito, 2003). These chemicals are rapid and have high percentage recovery of metals from herbal extracts. The certified 1000 ppm standard reference solution of each metal of interests was used. All glass wares were washed, placed in 20% ultrex nitric acid for 48 hours and rinsed with distilled water (Sahito, 2003).

3.8.3 Acid digestion of the plant extracts

The extracts were digested by acid oxidation method. The 0.5 g in Table 3.2 of each extract was digested by adding 1 ml of hydrogen peroxide (H_2O_2) together with 5 ml of HNO_3 in a 100 ml Pyrex beaker (Chandaka, 2017). The solution was heated on a hot plate at 95^0C for 15 minutes. Upon complete digestion, the solutions were allowed to cool, filtered and then put into volumetric flask of 50ml. Finally, the volume was toped up to 50ml by adding deionized water (Sahito, 2003). Each sample was kept in a refrigerator at -20° C till when needed for analyzes.

Table 3.2: Weight of extracts used for acid digestion

S/N	Plant extracts	Weight taken (g)
1	*Z. usamberense*	0.5
2	*Z. chalybeum*	0.5
3	*A. deserti*	0.5

3.8.4 Sample analysis

Acid digests of extracts was used to analyze metals such as K, Ca, Zn, Cu, Fe, Mg, Cr, Sr, Mn, As, V, Hg and Pb by flame AAS (Neetu, 2014). Upon setting the AAS, four standard solutions were aspirated into the flame to test the stability of the

working conditions (Ram, 2011). This was followed by flushing deionized water into the flame to set zero absorbance (Sahito, 2003). For metal analysis in the extracts, the above protocol was carried out four times. Mean absorbance were recorded and values obtained by using expression: (Amount of metal = C $\times$D/W) where, C = concentration, D = dilution factor and W = weight of extract. Values were given as μg/g (Ram, 2011).

3.9 Micro-organisms for bioassays

The standard microorganisms (Table 3.3) used in the study were obtained from stock cultures of Centre for Microbiology Research of Kenya Medical Research Institute (KEMRI), Kenya based on their reduced susceptibility to antibiotics (Oyetuuji, 2012) or resistant to antibiotics such as β-lactam agents (Olufunmiso, 2012). Microbes were stored in refrigerator at 4°C till used for *in vitro* studies.

Table 3.3: Standard micro-organisms used in the study

Bacterial strains	Code number
Gram-positive	
Bacillus cereus	ATCC 10876
Bacillus subtilis	ATCC 6633
Staphylococcus aureus	ATCC 29213
Enterococcus faecalis	ATCC 29912
Gram-negative	
Salmonella typhi	ATCC 19430
Escherichia coli	ATCC 25922
Shigella dysenteriae	ATCC 13313

American Type Culture Collection (ATCC)

3.10 Determination of antimicrobial activity

3.10.1 Preparation of 0.5 McFarland standard

McFarland Standard was made by adding 0.05ml of 1.175 % $BaCl.H_2O$ to 99.5 ml of 1 % H_2SO_4) (Appendix 2) with constant stirring. This gave approximately 1.5×10^8

cfu/ml cell density (Kitonde, 2013). The standard suspension was put in tube and tightly sealed to prevent evaporation (Kitonde, 2013).

3.10.2 Preparation of paper discs

The discs were prepared from filter papers (Whatman No.1) using a 6 mm diameter paper punch. The discs were sterilized by autoclaving for 15 minutes in sealed universal bottles (Kitonde, 2013).

3.10.3 Impregnation of paper discs

One gram of plant extract was mixed with in 1 ml of 0.5 % DMSO in bijou bottles. The discs (of 6mm diameter) were soaked in plant extract (India, 2005). Once seeded, discs were air dried for 5 minutes.

3.10.4 Inoculation of bacteria

Bacteria culture of each test bacteria was taken using sterilized wire loop and diluted in sterilized saline. The bacteria turbidity was compared to match with that of 0.5 McFarland standards which gives an equivalent density of 1.5×10^8 cfu/ml of bacteria (Omari, 2012). The bacteria culture was swabbed onto the prepared Mueller Hinton (MH) agar plates using sterilized cotton on wooden applicator (Kitonde, 2013).

3.10. 5 Placement of paper discs on plates

The air-dried discs were picked by a sterilized pair of forceps and aseptically placed gently onto MH agar plates (Omwenga, 2009). Antibiotic discs (Ciprofloxacin and Gentamycin) and discs soaked in DMSO were used as positive and negative control respectively. Ciprofloxacin and Gentamycin are associated with the least

antimicrobial resistance and are used for conventional treatment of bacterial infections (Chan, 2003). The plates were placed upside down in an incubator set at 37° C for 24 hours. The set-ups were repeated three times to ensure that results are reproducible.

3.10.6 Determination of inhibition activity.

The activity was assessed by measuring inhibition diameter (mm) of each bacterium using a transparent ruler in millimeter (Ogunshe, 2004) and compared with that of negative and positive controls.

3.11 Determination of Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) values

Serial dilution technique was employed to determine MIC of plant extracts against test microorganisms as reported by Clinical Laboratory Standard Institute (CLSI) (2008). The test was carried in 96 well micro-titer plates (U-shaped wells). The 100 µl of the extract was dispersed into the first well of micro-titer plate. The extract was serially diluted making the next well concentration half of that in the previous well (Omari, 2012). The concentrations were achieved by adding 50 µl of MH broth and transferring extract from the first well, throughout up to the last well (Omari, 2012). The impregnated discs (6mm) were picked using sterilized pair of forceps and transferred to MH agar on petri-dish plates smeared with 1.5×10^{8} cfu/ml of test microorganisms (Kitonde, 2013). These plates were incubated at 37°C and observation recorded after 24 hours (Jiun, 2014). All set-ups were performed in triplicates. Based on this study, the MIC is the minimal concentration of plant extracts that indicated no detectable inhibition of the test microbes (Omari, 2012).

Subsequently, the MBC was determined to establish the lowest concentration at which plant extract kills a particular microorganism. The 10 µl of bacteria suspensions (1.5×10^8 cfu/ml density) was put into each extract concentration in 96 well micro-titer plates (Kitonde, 2013) and incubated at 37℃ for 4 hours. All tests were executed in triplicates to validate results. Ultimately, bacterial growth was detected by streaking each bacterium evenly onto prepared nutrient agar using sterilized wire loop and further incubated, 24 hours at 37°C (Jiun, 2014). Absence of bacterial growth confirmed the killing of the bacteria (Bactericidal). Plate with growth confirmed that extract only inhibited growth of bacteria (Bacteriostatic). The MBC is the lowermost concentration of extracts which produces no colony growth (Kitonde, 2013).

3.12 Time kill studies

3.12.1 Test cultures

The time kill activity was assessed against *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29912 and *Escherichia coli* 35218 based on their MIC values.

3.12.2 Time kill assay

Herbal extracts were tested for time kill against the test microorganisms based on their MIC endpoints. The MICs endpoints were used as a guide to assess the speed of bactericidal activity (Olila, 2001; Oladosu, 2013). The inoculum culture of test microorganisms (*B. cereus* ATCC 10876, *B. subtilis* ATCC 6633, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29912, *E. coli* ATCC 25922) containing approximately 1.5×10^6cfu/ml (adjusted McFarland) (Appendix 2) bacterial cells were used in these bioassays (Olila, 2001). The activities were performed in glass tubes containing 9 ml

of the plant extracts in Mueller Hinton broth at 0.5×MIC, 1×MIC, and 2×MIC concentrations.

An inoculum suspension of 1 ml was introduced into Mueller Hinton broth containing plant extracts and incubated at 37℃ for 90 minutes in an orbital shaker at 100 rpm to ensure that organisms occupy their logarithmic phases. The 10 µl of the mixture was removed and plated at varying time intervals of 0, 2, 4, 6, 8 and 24 h onto Nutrient Agar plates in triplicates. The control set-up (plant extracts free Nutrient Agar - growth) was seeded with test inoculums. The plates were incubated overnight at 37℃ for 24 hours. The plate count method was used to count the number of viable cells (Oladosu, 2013) and only counts falling in between 30-300 for each dilution were recorded. The percentage kill or growth of bacteria (Olila, 2001) was calculated as follows;

$$\%\text{ of organisms killed or grown} = \left(1 - \frac{\text{CFUs/ml at } \times \text{time interval}}{\text{CFUs/ml at 0 hour}}\right) \times 100$$

3.12.3 Time kill endpoints

The time kill endpoints involved actual reduction of viable CFUs/ml as described by Oladosu (2013). Bactericidal activity refers to viable cells reduction by 99.9 % kill over the specified time period (Oladosu, 2013), whereas bacteriostatic activity refers to viable cells increase compared to a starting inoculum (Peterson, 2007). The constant rate of logarithmic kill was assumed during the time kill study (Ojo, 2013).

3.13 *In vivo* toxicity studies

3.13.1 Preparation of doses for toxicity

The male mice weighing 23 grams was exposed to 450, 670 and 1000 mg/kg body weight/day of extract. Dose of plant extract was prepared by dissolving 1.449 g in 2ml (450 mg/kg body weight), 2.157 g in 2ml (670 mg/kg body weight) and 3.22 g in 2ml (1000 mg/kg body weight) (Appendix 1). Herbal drug of 0.1ml was administered animals daily (Table 3.4) for 28 days.

3.13.2 Experimental design (grouping and dosing of animals)

Animals were procured from Kenya Medical Research Institute (KEMRI), Kenya. After 7 days of acclimatization, animals were put into two categories (oral and intraperitoneal), each comprising of four groups of 5 mice (Table 3.4).

Table 3.4: Experimental design for toxicity

Route of administration	5 animals per groups	Treatment (mg/kg bw)	Duration (days)
Oral	1	Control (0.1ml Normal saline)	28
	2	450	28
	3	670	28
	4	1000	28
Intraperitoneal	1	Control (0.1ml Normal saline)	28
	2	450	28
	3	670	28
	4	1000	28

The first group of each category was given 0.1ml of normal saline as control for 4 weeks. The remaining groups for both oral and intraperitoneal study, animals were given varying dosage (450, 670, and 1000 mg/kg body weight/day) (Hayelom, 2011). During this period, mice were housed 5 per cage under good hygiene and natural light/dark cycles. They were maintained on standard pellets accompanied with water and monitored for any symptoms of sickness and mortality (Teshager, 2016).

3.13.3 Absolute weight of the body

All animal weights were estimated after every week using a digital electronic balance (Sartorius AG) including the 28th day of the animal sacrifice (Mosaid, 2012).

3.13.4 Animal dissection and measurement of organ weight

Animals of each group were euthanized by use of chloroform and sacrificed at the end of 28th day (Abiba, 2013). The kidney, liver, brain, testes, lungs, heart and spleen were removed and weighed in grams. The relative percentage organ weight to body weight (ROW) (Aniagu, 2005) was then computed as follows:

$$\text{Relative \% organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of mice on sacrifice day (day)}} \times 100$$

3.14 Acquiring sera samples

Blood was taken from the heart of every mouse on Day 28. The blood was collected with aid of 5ml syringe and put into two portions (Abiba, 2013). Blood was placed in heparinized EDTA capped bottles for hematological analysis. The other sample was allowed to clot. The clotted blood was rotated at high speed of 3000 rpm for 10 minutes to separate serum from solids. The obtained serum was stored frozen at -20℃ in non-heparinized capped bottles till used for the assay of serum chemistry (Aniagu, 2005).

3.14.1 Determination of Hematological Parameters

Red blood cells, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, red cell distribution width, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, platelets distribution width and mean platelets

volume were determined using the Coulter Counter System Beckman Coulter® (ThermoFisher, UK) (Abiba, 2013). Eosinophils, Monocytes, Lymphocytes, Neutrophils and Basophils (Cells $\times 10^3$) μL^{-1} counts were attained by calculating their percentage differential count against the total WBC absolute count (Aniagu, 2005) and compared with normal reference values (CDC, 2018) (Appendix 4).

3.14.2 Determining Enzyme Activities

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), gamma glut amyl transferase (GGT), lactate dehydrogenase (LDH), α-amylase, Creatine kinase (CK), Glucose (GLU), direct bilirubin (D-BIL), total bilirubin (T-BIL), indirect bilirubin (I-BIL), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) were tested using the automated Olympus 640 chemistry Auto Analyzer. All tests were carried out using the standard operating methods printed and kept in Laboratory Medicine Department, Kenyatta National Hospital, Kenya (Murugi, 2012; Odongo, 2013).

3.14.2.1 Determination of Alanine Aminotransferase activity (ALT)

The α-ketoglutarate reacts with L-alanine in the presence of alanine aminotransferase to form pyruvate and L-glutamate. The pyruvate was reduced to give lactate in the presence of lactate dehydrogenase (LDH), followed by oxidation of β-Nicotinamide Adenine Dinucleotide (NADH) to give β-Nicotinamide Adenine Dinucleotide (NAD) (Abiba, 2013). The serum (10 µl) was mixed reagent (110 µl). Absorbed light was monitered at 340 nm which was directly proportional to alanine aminotransferase

activity (Murugi, 2012). Autoanalyzer expressed the activity in IU/L. The enzymatic breakdown occured at 37°C for three minutes.

Summary of methodology:

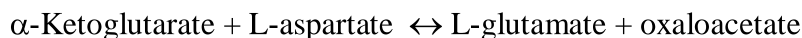
α - Ketoglutarate + L-alanine ↔ Pyruvate + L-glutamate

Pyruvate + NADH + H^+ ↔ Lactate + NAD^+

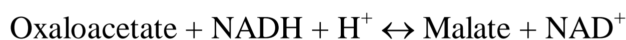
3.14.2.2 Determination of Aspartate aminotransferase activity (AST)

The α-ketoglutarate reacts with L-aspartate in presence of aspartate aminotransferase to give L-glutamate and oxaloacetate. The malate dehydrogenase reduced oxaloacetate to give malate, followed by brekdown of β-nicotinamide adenine to dinucleotide (Abiba, 2013). The serum (10 µl) was mixed with the reagent (110 µl). Absorbed light was monitered at 340 nm which was directly proportional to aspartate aminotransferase activity (Murugi, 2012). Aauto-analyzer expressed the activity in IU/L. The enzymatic breakdown occurred occured at 37°C for three minutes.

Summary of methodology:



α-Ketoglutarate + L-aspartate ↔ L-glutamate + oxaloacetate

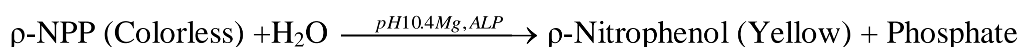


Oxaloacetate + NADH + H^+ ↔ Malate + NAD^+

3.14.2.3 Determination of Alkaline Phosphatase activity (ALP)

The colorless ρ-nitrophenylphosphate (pNPP) reacts with alkaline phosphatase in presence of Mg^+ to form phosphate and ρ-nitrophenol (yellow) at pH 10.4 (Abiba, 2013). The five microliter of the serum was mixed with 250 µl of the reagent. Absorbed light was monitered at 410 nm which was directly proportional to alkaline phosphatase activity (Murugi, 2012). Auto-analyzer expressed the activity in IU/L. The enzymatic breakdown occurred occured at 37°C for three minutes.

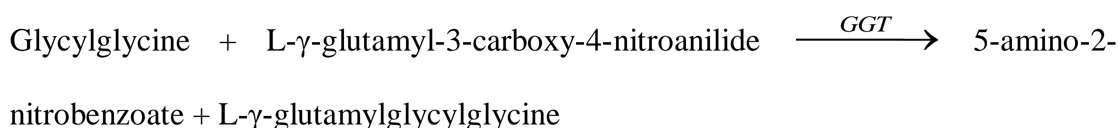
Summary of methodology:


ρ-NPP (Colorless) +H_2O $\xrightarrow{pH10.4Mg,ALP}$ ρ-Nitrophenol (Yellow) + Phosphate

3.14.2.4 Determination of gamma glutamyl transferase activity (GGT)

L-γ-glutamyl-3-carboxy-4-nitroanilide reacts with glycylglycine in presence of gamma glutamyl transferase to form glutamylglycylglycine and 5-amino-2-nitrobenzoate (Abiba, 2013). Five microliters of the serum were mixed with 200 µl of the reagent. Absorbed light was monitered at 405 nm which was directly proportional to γ-glutamyl transferase activity (Murugi, 2012). Auto-analyzer expressed the activity in IU/L. The enzymatic breakdown occurred at 37°C for three minutes.

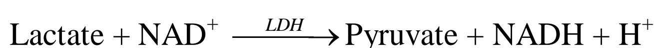
Summary of methodology:


Glycylglycine + L-γ-glutamyl-3-carboxy-4-nitroanilide $\xrightarrow{GGT}$ 5-amino-2-nitrobenzoate + L-γ-glutamylglycylglycine

3.14.2.5 Determination of Lactate Dehydrogenase activity (LDH)

The oxidation of lactate in presence of Lactate Dehydrogenase forms pyruvate followed by the reduction of NAD^+ to NADH. Two microliter of serum was reacted with 40 µl of the reagent. Absorbed light was monitered at 340 nm which was directly proportional to lactate dehydrogenase activity (Murugi, 2012). Auto-analyzer expressed the activity in IU/L. The enzymatic breakdown occurred at 37°C for three and half minutes.

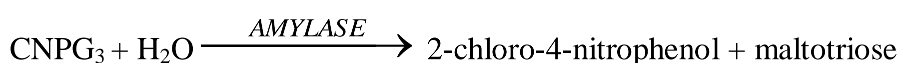
Summary of methodology:


Lactate + NAD^+ $\xrightarrow{LDH}$ Pyruvate + NADH + H^+

3.14.2.6 Determination of α-Amylase activity (α-AMYL)

The α-Amylase reacts with 2-chloro-4-nitrophenyl-α-D-maltotrioside ($CNPG_3$) giving 2-chloro-4-nitrophenol. Three microliter of serum was reacted with 300 µl of the reagent. Absorbed light as monitored at 340 nm which was directly proportional to α-Amylase activity (Gaelle, 2020). Auto-analyzer expressed the activity in IU/L. The enzymatic breakdown occurred at 37°C for three minutes.

Summary of methodology:

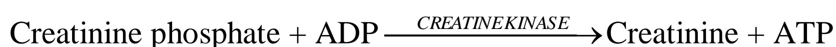


$$CNPG_3 + H_2O \xrightarrow{AMYLASE} \text{2-chloro-4-nitrophenol + maltotriose}$$

3.14.2.7 Determination of Creatine kinase activity (CK)

The adenosine diphosphate (ADP) reacts with Creatine phosphate in presence of Creatine kinase to form creatine and adenosine triphosphate (ATP) (Abiba, 2013). One microliter of the serum was mixed with 50 µl of the reagent. Absorbed light was monitored at 340 nm which was directly proportional to Creatine kinase activity (Murugi, 2012). Auto-analyzer expressed the activity in IU/L. The enzymatic breakdown occured at 37°C for three minutes.

Summary of methodology:



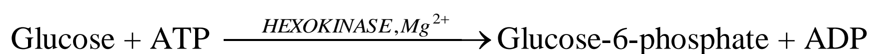
$$\text{Creatinine phosphate + ADP} \xrightarrow{CREATINEKINASE} \text{Creatinine + ATP}$$

3.14.2.8 Determination of glucose (GLU)

Glucose is broken in the presence of adenosine triphosphate (ATP) and Mg^{2+} by hexokinase to produce adenosine diphosphate and glucose-6-phosphate (G-6-P). Glucose-6-phosphate dehydrogenase (G-6-P DH) oxidizes G-6-P to 6-Phosphogucono-δ-lactone and reduces $NADP^+$ to NADPH. One microliter of the serum was mixed with 50 µl of the reagent. Absorbed light was monitered at 340 nm

which was directly proportional to glucose activity. Auto-analyzer expressed activity in mm/L (Soetan, 2010). The enzymatic breakdown occured at 37°C for three minutes.

Summary of methodology:



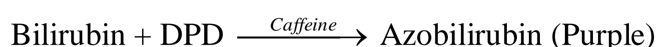
$$\text{Glucose} + \text{ATP} \xrightarrow{HEXOKINASE, Mg^{2+}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{NADP}^{+} + \text{G-6-P} \xrightarrow{G6PDH} \text{6-Phosphoglucono-}\delta\text{-lactone} + \text{NADPH}$$

3.14.2.9 Determination of total bilirubin (T-BIL)

Conjugated bilirubin and unconjugated bilirubin were reacted with 3, 5-dichlorophenyl diazonium tetrafluoroborate (DPD) in the presence of an accelerator (caffeine) to form azobilirubin (Soetan, 2010). A sample of 8 µl was reacted with 280 µl of reagent. Absorbed light was monitored at 552 nm which was directly proportional to T-BIL activity**.** Auto-analyzer expressed activity in µmol/L. The enzymatic breakdown occured at 37°C for two minutes.

Summary of methodology:

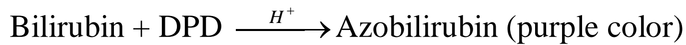


$$\text{Bilirubin} + \text{DPD} \xrightarrow{Caffeine} \text{Azobilirubin (Purple)}$$

3.14.2.10 Determination of direct bilirubin (D-BIL)

Conjugated bilirubin was reacted with stabilized diazonium salt (3, 5-dichlorophenyldiazonium tetra-fluoroborate (DPD) in an acidic medium to form Azobilirubin. A sample of 5 µl was reacted with 160 µl of reagent. Absorbed light was monitored at 546nm which was directly proportional to D-BIL activity (Soetan, 2010). Auto-analyzer expressed activity in µmol/L. The enzymatic breakdown occured at 37°C for two minutes.

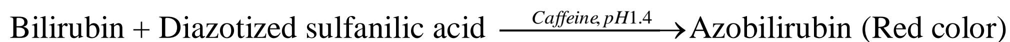
Summary of methodology:



3.14.2.11 Determination of indirect bilirubin (I-BIL)

Unconjugated bilirubin was reacted with stabilized diazotized sulfanilic acid to form azobilirubin in the presence of caffeine. A sample of 5 µl was reacted with 160 µl of reagent. Absorbed light was monitored at 578nm which was directly proportional to I-BIL activity (Soetan, 2010). Auto-analyzer expressed concentration in µmol/L. The enzymatic breakdown occured at 37°C for two minutes.

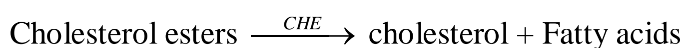
Summary of methodology:

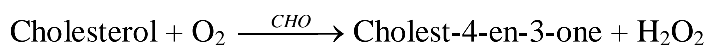


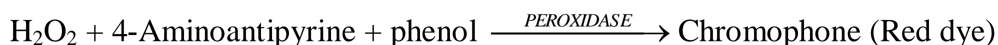
3.14.2.12 Determination of total cholesterol (T-CHOL)

Serum cholesterol esters are hydrolyzed by cholesterol esterase (CHE) to free cholesterol and free fatty acids. Cholesterol oxidase (CHO) oxidizes free cholesterol to cholest-4-en-3-one with simultaneous production of hydrogen peroxide (H_2O_2). Hydrogen peroxide reacts with 4-aminoantipyrine and phenol cataltzed by peroxidase to form chromophone. A sample of 5 µl was reacted with 160 µl of reagent. Absorbed light was monitored at 540nm which was directly proportional to T-CHOL activity (Soetan, 2010). Auto-analyzer expressed activity in mm/L. The enzymatic breakdown occured at 37°C for two minutes.

Summary of methodology:







3.14.2.13 Determination of high-density lipoprotein cholesterol (HDL-CHOL)

Total serum HDL-cholesterol is specifically quantified using the same method used in the estimation of total cholesterol but initially the other lipoproteins are removed from the serum sample. This is achieved by the addition of phosphotungstic acid and magnesium ions which promote the precipitation of LDL, VLDL and Chylomicrons. Following centrifugation, the cholesterol content of the HDL-containing supernatant is assayed. The HDL-cholesterol was hydrolyzed by cholesterol esterase, oxidized by cholesterol oxidase to choleterone which reacts with a chromogen to yield a blue color complex. A sample of 5 µl was reacted with 160 µl of reagent. Absorbed light was monitored at 600nm which was directly proportional to HDL-CHOL activity (Soetan, 2010). Auto-analyzer expressed activity in mmol/L. The enzymatic breakdown occured at 37°C for two minutes.

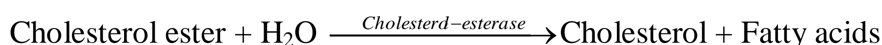
Summary of methodology:

HDL-cholesterol $\xrightarrow{CHE, CHO \& Chromogen system}$ Blue color complex

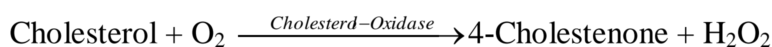
3.14.2.14 Determination of low-density lipoprotein cholesterol (LDL-C)

Cholesterol esterase (CHE) and cholesterol oxidase (CHO) hydrolyse and oxidase, respectively, serum cholesterol esters at pH 7.0 to cholestenone and hydrogen peroxide (H_2O_2). Surfacants reacts with cholestenone giving 4-aminoantipyrine (4AA) and 3, 5-Dimethoxy-N-ethyl-N-(2-hydroxy-3-sulfopropy1) aniline (HDAOS) aniline derivate. In presence of peroxidase, hydrogen peroxide condenses HDAOS and 4AA to form a quinoneimine (red dye). A sample of 5 µl serum was reacted with 160 µl of reagent. Absorbed light was monitored at 540nm which was directly proportional to LDL-CHOL activity (Soetan, 2010). Auto-analyzer expressed the activity in mmol/L. The enzymatic breakdown occured at 37°C for two minutes.

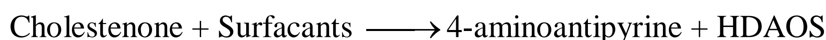
Summary of methodology:



Cholesterol ester + H_2O $\xrightarrow{Cholesterd-esterase}$ Cholesterol + Fatty acids



Cholesterol + O_2 $\xrightarrow{Cholesterd-Oxidase}$ 4-Cholestenone + H_2O_2



Cholestenone + Surfacants $\longrightarrow$ 4-aminoantipyrine + HDAOS

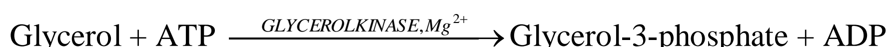
H_2O_2 + 4-aminoantipyrine + HDAOS $\xrightarrow{Peroxidase}$ Quinoneinine (Red dye)

3.14.2.15 Determination of triglyceride (TG)

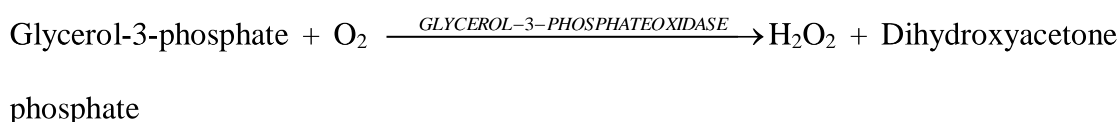
Serum triacylglycerols are hydrolysed by lipase to glycerol and fatty acids. Adenosine triphosphate (ATP) phosphorylates glycerol giving glycerol-3-phosphate in a reaction catalyzed by glycerol kinase. In the presence of glycerol 3-phosphate oxidase (GPO), glycerol-3-phosphate was oxidized to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). And in the presence of peroxidase (POD), hydrogen peroxide reacts with 4-aminophenazone (AAP) and (4-sulfobutyl)-3, 5-dimethylaniline, disodium salt (MADB) to produce a chromophone. A sample of 5 µl serum was reacted with 160 µl of reagent. Absorbed lght was monitored at 880nm which was directly proportional to TG activity (Soetan, 2010). Auto-analyzer expressed the activity in mmol/L. The enzymatic breakdown occured at 37°C for two minutes.

Summary of methodology:

Triglycerides + $3H_2O$ $\xrightarrow{LIPASE}$ Glycerol + 3Fatty acids



Glycerol + ATP $\xrightarrow{GLYCEROLKINASE, Mg^{2+}}$ Glycerol-3-phosphate + ADP



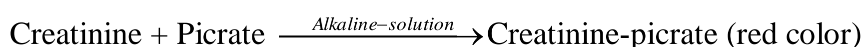
Glycerol-3-phosphate + O_2 $\xrightarrow{GLYCEROL-3-PHOSPHATEOXIDASE}$ H_2O_2 + Dihydroxyacetone phosphate

$2H_2O_2$ + MADB + AAP $\xrightarrow{PEROXIDASE}$ Blue dye + OH^- + H_2O

3.14.2.16 Determination of creatinine (CREAT)

Creatinine was reacted with picrate in an alkaline solution to form a creatinine-picrate complex (red). A sample of 20 µl was reacted with 220 µl of reagent. Absorbed light was monitered at 512 nm which was directly proportional to creatinine activity. Auto-analyzer expressed the activity in µmol/L (Soetan, 2010). The enzymatic breakdown occured at 37°C for one minute.

Summary of methodology:



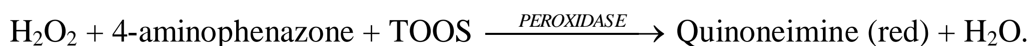
Creatinine + Picrate $\xrightarrow{Alkaline-solution}$ Creatinine-picrate (red color)

3.14.2.17 Determination of uric acid (UA)

Urease oxidizes uric acid to produce allantoin, hydrogen peroxide and carbon (IV) oxide (CO_2). In presence of peroxidase, hydrogen peroxide reacts with 4-aminophenazone and N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS sodium salt) giving quinoneimine. A sample of 3 µl serum was reacted with 300 µl of reagent. Absorbed light was monitored at 546nm which was directly proportional to urea activity (Soetan, 2010). Auto-analyzer expressed the activity in mmol/L. The enzymatic breakdown occured at 37°C for three and half minutes.

Summary of methodology:

Uric acid + O_2 + H_2O $\xrightarrow{UREASE}$ Allantoin + H_2O_2 +CO_2



H_2O_2 + 4-aminophenazone + TOOS $\xrightarrow{PEROXIDASE}$ Quinoneimine (red) + H_2O.

3.15 Data management and statistical analysis

Results were initially recorded in the laboratory notebook. Results were then entered into the Excel Spreadsheet and cleaned for errors and exported to SPSS software and subjected to descriptive statistical analysis. Descriptive statistic results were expressed

as Mean ± SD (Standard Deviation). Results were presented in Figures and Tables for comparing animal weights in each of the four dose groups in four weeks. Independent one-way ANOVA and Tukey's method was carried out to differentiate means of control and treated groups (Al-Ostoot, 2018). A ρ-value of less or equal to 0.05 was considered significant.

CHAPTER FOUR

RESULTS

4.1 Extracts of medicinal plants

The results shown in Table 4.1 are the weight in grams of dry powder and percentage yield of the extract. The methanol yield extract obtained weighed 24 g of stem bark of *Z. usamberense* (dark semi-solid substance), 18 g of stem bark of *Z. chalybeum* (brown semi-solid substance) and 15 g of leaf of *A. deserti* (dark semi-solid substance) all with herbal smell. The methanol extraction of *Z. usamberense* plant yielded the highest percentage yield of 16 %, *Z. chalybeum* had yield of 12 % and *A. deserti* had the lowest yield of 10 %.

Table 4.1: The plants extract yields (g) of each plant materials

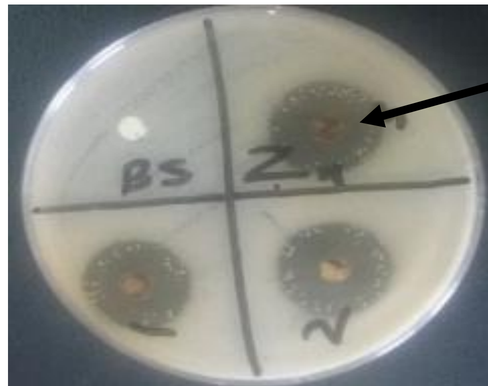
Plant species	Part used	Dry powder (g)	Yield (g)	Yield (%)
Zanthoxylum usamberense	Stem bark	150	24	16
Aloe deserti	Leaf	150	18	12
Zanthoxylum chalbeum	Stem bark	150	15	10

4.2 *In vitro* antimicrobial assay

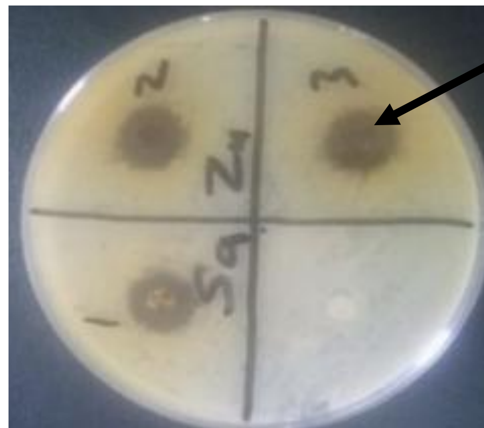
The clear area in Plate 4.1 around the paper disk indicated growth inhibition against test micro-organisms. The *Z. usamberense* methanol extract demonstrated the highest growth inhibition against *B. subtilis* ATCC 6633, *S. aureus* ATCC 29213 and *B. cereus* ATCC 10876 compared to controls. Similarly, *Z. usamberense* methanol extract in Plate 4.1 indicated low inhibition of *E. faecalis* ATCC 29912 and *E. coli* ATCC 25922 compared to positive controls. The *S. tyhpi* ATCC 19430 and *S. dysentireae* ATCC 13313 showed resistance to *Z. usamberense* methanol extract. Methanol extract of *Z. chalybeum* plant inhibited growth of *B. subtilis* ATCC 6633 in Plate 4.2. The *Z. chalybeum* methanol extract indicated weak inhibition to the growth of *E. faecalis* ATCC29912, *B. cereus* ATCC 10876, and *S. aureus* ATCC 29213. All

the tested Gram-negative bacteria indicated resistance to *Z. chalybeum* methanol extract. Methanol extract of *A. deserti* showed no inhibition to all test microbes. In positive controls, Ciprofloxacin and Gentamycin in Plate 4.3 showed high inhibitory activities of *E. faecalis* ATCC 29912, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *S. aureus* ATCC 29213, *S. dysentireae* ATCC 13313, *S. typhi* ATCC 19430 and *E. coli* ATCC 25922.

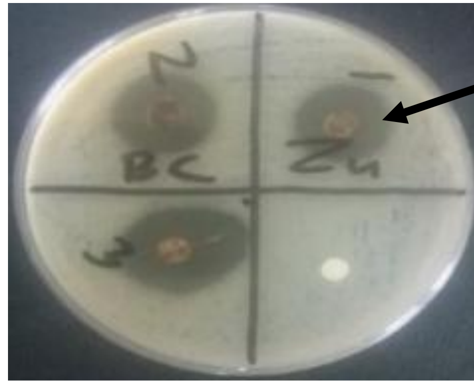
Plate 4.1: *Z. usamberense* extract inhibition against test micro-organisms



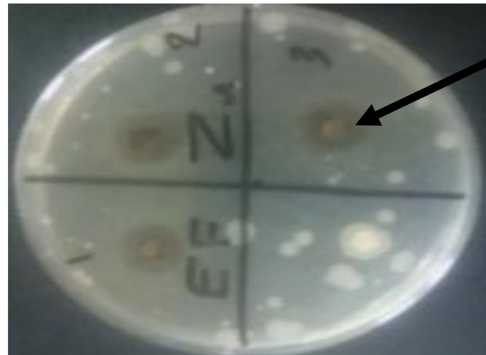
Inhibition zone exhibited by *Z. usamberense* extract (100 mg/ml) against *B. subtilis* ATC C6633



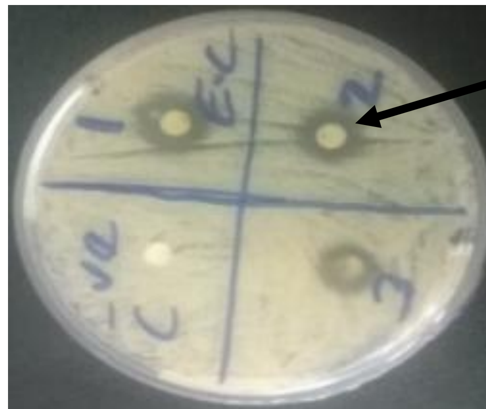
Inhibition zone exhibited by *Z. usamberense* extract (100 mg/ml) against *S. aureus* ATCC 29213



Inhibition zone exhibited by *Z. usamberense* extract (100 mg/ml) against *Bacillus cereus* ATCC 10876

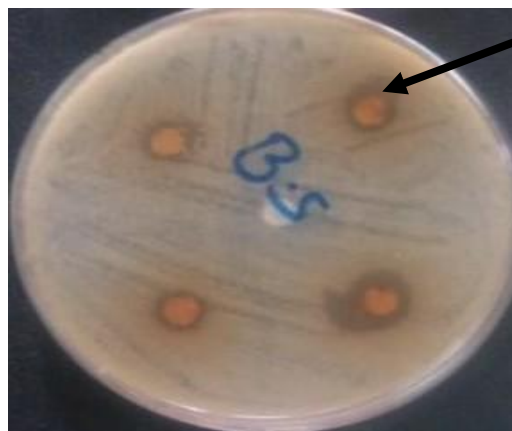


Inhibition zone exhibited by *Z. usamberense* extract (100 mg/ml) against *E. faecalis* ATCC 29912



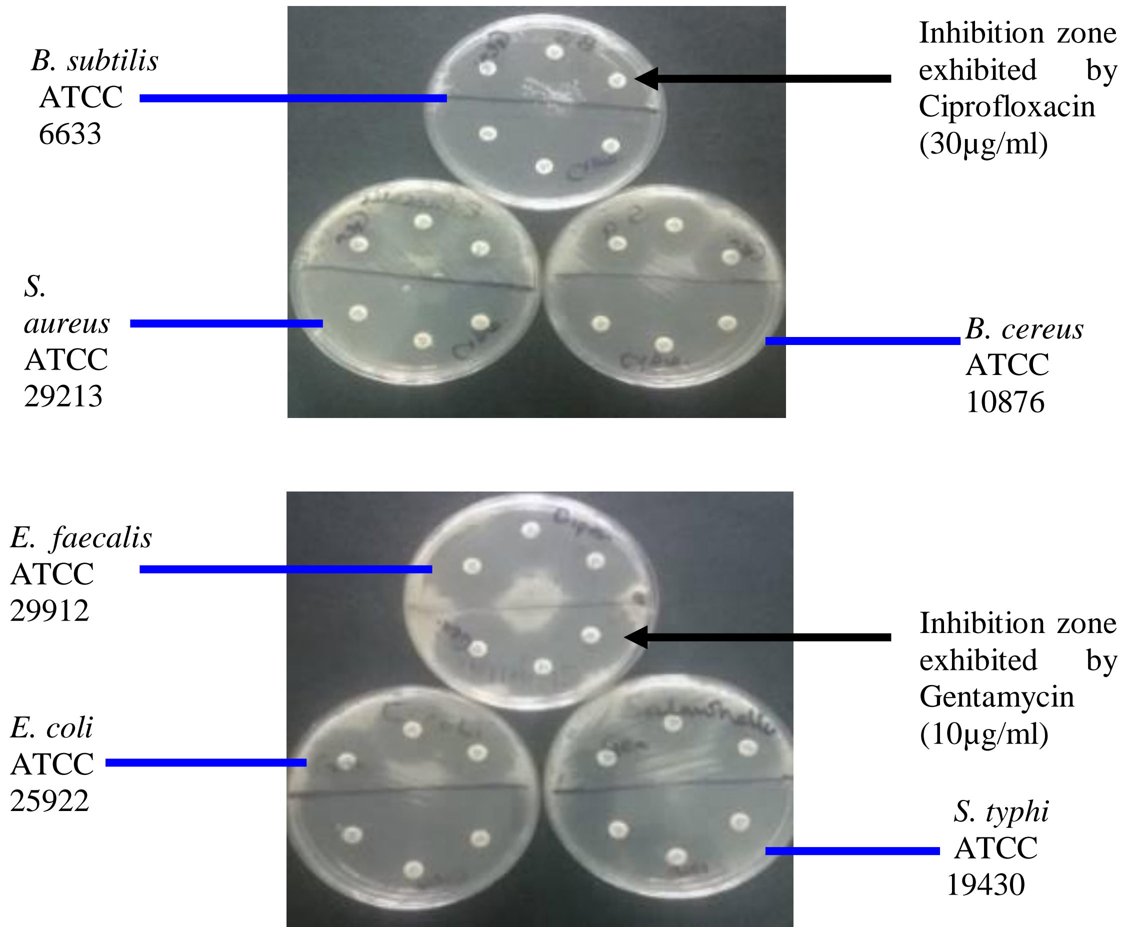
Inhibition zone exhibited by *Z. usamberense* extract (100 mg/ml) against *E. coli* ATCC 25922

Plate 4.2: *Z. chalybeum* extract inhibition against test micro-organism



Inhibition zone exhibited by *Z. chalybeum* extract (100 mg/ml) against *B. subtilis* ATCC 6633

Plate 4.3: Antibiotics inhibition against test micro-organisms



Methanol extracts of *Z. usamberense* in Table 4.2 showed significant ($\rho \leq .05$) inhibition zones of *B. subtilis* ATCC 6633 (29.00 ± 0.82 mm), *B. cereus* ATCC 10876 (22.67 ± 2.05 mm) as compared to the positive controls and low inhibition in *S. aureus* ATCC 29213 (14.00 ± 0.82 mm), *E. coli* ATCC 25922 (10.33 ± 1.25 mm) and *E. faecalis* ATCC 29912 (13.00 ± 0.82 mm). Similarly, methanol stem bark extract of *Z. chalybeum* in Table 4.2 showed significant ($\rho \leq .05$) inhibition zone of *B. subtilis* ATC C6633 (16.00 ± 0.82 mm) and low/weak inhibition in *B. cereus* ATCC 10876 (7.67 ± 1.25 mm).

As shown in Table 4.2, Ciprofloxacin (30 µg/ml) gave diameter of inhibition of *E. faecalis* ATCC 29912 (33.67 ± 1.25 mm), *B. subtilis* ATCC 6633 (36.33 ± 1.25 mm), *B. cereus* ATCC 10876 (36.33 ± 1.25 mm), *S. aureus* ATCC 29213 (36.33 ± 1.25 mm) and *Escherichia coli* ATCC 25922 (37.00 ± 0.82 mm). Gentamycin (10 µm/ml) gave diameter of inhibition of *E. faecalis* ATCC 29912 (17.00 ± 0.82 mm), *B. subtilis* ATCC 6633 (29.00 ± 0.82 mm), *B. cereus* ATCC 10876 (29.00 ± 0.82 mm), *S. aureus* ATCC 29213 (29.00 ± 0.83 mm) and *E. coli* ATCC 25922 (29.67 ± 0.94 mm). The positive control showed significant zones of growth inhibition but there was no inhibitory activity of DMSO used as negative controls. The zone of inhibition of between 7 mm and 10 mm was considered as weak activity, between 11 mm and 14 mm was considered as moderate activity and those of from 15 mm and above were considered as high activity.

Table 4.2: Diameter of antibacterial inhibition zones of test microbes

		Methanol extract (100mg/ml)				
		Zone of inhibition (mm) and standard deviation (SD)				
Name of organism	Number	*Zanthoxylum usamberense* (100mg/ml)	*Zanthoxylum chalybeum* 100mg/ml)	Positive control (Ciprofloxacin) (30mg/ml)	Positive control (Gentamycin) (10mg/ml)	Negative control (DMSO)
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Escherichia coli	ATCC 25922	12.00±0.82[a]	ND	37.00±0.82	29.67±0.94	-
Bacillus subtilis	ATCC 6633	29.00±0.82[e]	16.00±0.82[b]	36.33±1.25	29.00±0.82	-
Bacillus cereus	ATCC 10876	21.00±0.82[d]	7.67±1.25[a]	36.33±1.25	29.00±0.82	-
Enterococcus faecalis	ATCC 29912	13.00±0.82[b]	ND	33.67±1.25	17.00±0.82	-
Staphylococcus aureus	ATCC 29213	14.00±0.82[c]	7.33±0.47[a]	36.33±1.25	29.00±0.82	-

Descriptive statistic results were reported as Means ± SD for the triplicates. Zones of inhibition in same column identified by different letters indicate significant differences at $\rho \leq 0.05$ when compared with Ciprofloxacin

ND = Note done, DMSO =Dimethyl sulphoxide

4.3 Minimum Inhibitory Concentration (MICs) values

The results shown in Plate 4.4 are inhibition zones produced by *Z. usamberense* methanol extract at varying concentration against the tested microorganisms. The data in Table 4.3 indicated that the extract inhibited the growth of the test microorganisms with MIC of 12. 5 mg/ml for *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876 and *S.* aureus ATCC 29213. *Z. usamberense* methanol extract demonstrated MIC of 25 mg/ml for *E. faecalis* ATCC 29912 and 50 mg/ml for *E. coli* ATCC 25922. In addition to this, *B. subtilis* ATCC 6633, *S. aureus* ATCC 29213 and *B. cereus* ATCC 10876 showed significant ($\rho \leq 0.05$) MIC activity that was more susceptible at the lowest concentration of 12.5mg/ml compared to other bacterial species.

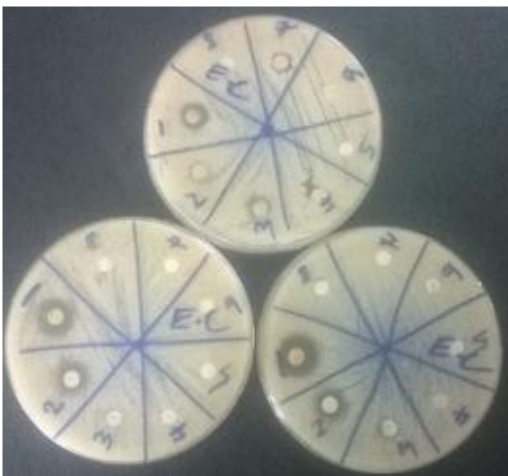
Plate 4.4: MICs of *Z. usamberense* methanol extract

Against *S. aureus* ATCC 29213

Against *B. subtilis* ATC C6633

Against *E. faecalis* ATCC 29912

Against *B. cereus* ATCC 10876



Against *E. coli* ATCC 25922

Table 4.3: The MICs values of *Z. usamberense* against test microbes

No	Microorganism	Extract concentration (mg/ml) /Zone of inhibitions (mm)					
		Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	
		100	50	25	12.5	6.25	MIC (mg/ml)
1	*E. faecalis* ATCC 29912	13.00±0.82[b]	10.33±1.70[a]	6.67±0.67[a]	-	-	25.0
2	*B. cereus* ATCC 10876	22.67±2.05[e]	18.00±1.63[c]	12.67±2.5[b]	9.00±3.56[a]	6.33±0.47[a]	12.5
3	*B. subtilis* ATCC 6633	29.00±0.82[d]	17.67±0.47[c]	14.33±0.47[c]	10.67±1.45[a]	6.33±0.47[a]	12.5
4	*S. aureus* ATCC 292132	14.00±0.82[c]	11.33±1.70[b]	9.67±0.94[a]	8.00±0.00[a]	-	12.5
5	*E. coli* ATCC 25922	10.33±1.25[a]	9.00±1.82[a]	-	-	-	50

Descriptive statistic results were reported as Means ± SD for the triplicates. Zones of inhibition in same column identified by different letters indicate significant differences at $\rho \leq 0.05$.

4.4 The Minimum Bactericidal Concentration (MBCs) values

The results shown in Table 4.4 are MBCs values produced by *Z. usamberense* methanol extract against test microorganisms. The MBCs values were 12.5 mg/ml for *B. cereus* ATCC 10876, 12.5 mg/ml for *B. subtilis* ATCC 6633 and 50 mg/ml for *E. faecalis* ATCC 29912, 25 mg/ml for *S. aureus* ATCC 29213 and 100 mg/ml for *E. coli* ATCC 35218.

Table 4.4: The MBC of *Z. usamberense* methanol extract

	Strains of bacteria	**Methanol extract concentration (mg/ml)**					
		100	50	25	12.5	6.25	MBC (mg/ml)
1	*E. faecalis* ATCC 29912	NG	NG	G	G	G	50.00
2	*B. cereus* ATCC 10876	NG	NG	NG	NG	G	12.5
3	*B. subtilis* ATCC 6633	NG	NG	NG	NG	G	12.5
4	*S. aureus* ATCC 29213	NG	NG	NG	G	G	25
5	*E. coli* ATCC 25922	NG	G	G	G	G	100

Legend:
G - Growth, NG – No growth, MBC – Minimum bactericidal concentration

4.5 The MIC and MBC for methanol extract of *Z. usamberense*

The results shown in Table 4.5 are MBC/MIC ratios produced by *Z. usamberense* methanol extract against test micro-organisms. The MBC/MIC ratios were 2.0 for *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29912, 1.0 for *B. subtilis* ATCC 6633 and *B. cereus* ATCC 10876. The bacteria *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29213 had the highest MBC/MIC value of 2.0 compared to other bacteria species.

Table 4.5: The MBCs /MICs values of *Z. usamberense* methanol extract

Reference bacteria	**MIC**	**MBC**	**MBC/MIC (Ratios)**
Staphylococcus aureus ATCC 29213	12.5	25.0	2.0
Bacillus subtilis ATCC 6633	12.5	12.5	1.0
Enterococcus faecalis ATCC 29912	25.0	50.00	2.0
Bacillus cereus ATCC 10876	12.5	12.5	1.0
Escherichia coli ATCC 25922	50.00	100.00	2.0

4.6 *In vitro* time-kill kinetics of *Z. usamberense* extract

The results shown in Table 4.6 are the time-kill kinetics produced by methanol extract of *Z. usamberense* against *B. cereus* ATCC 10876. In the growth control, colonies increased sharply from initial colonies to 246.60 ± 5.25 CFUs/ml after 4 hr and became too numerous to count after 24 hr. In the extracts concentration 0.5×MIC, there was an abrupt reduction in the bacteria colonies to 74.33 ± 4.04 CFUs/ml after 2 hr. The percentage kill of viable colonies was observed to be from 53.1 % within the 2hr of incubation. However, the colonies increased steadily to 230.00 ± 10.00 CFUs/ml and later became too numerous to count after 24 hr. In the extracts concentration 1×MIC, there was an abrupt decline in the colonies after 8hr to 8.00 ± 2.00 CFUs/ml. Finally, rapid killing of colonies was 99.9 % at 24 hr indicating a significantly high reduction compared to positive control ($p \leq .05$). This gave total bacteria colonies elimination by the plant methanol extracts after 24 hr exposure time as indicated in Table 4.6. In the extracts concentration 2×MIC, there was a reduction from original colonies to 41.67 ± 2.89 CFUs/ml after 2 hr, which kept on reducing to 5.00 ± 2.00 CFUs/ml after 8 hr. Finally, rapid killing of the entire colonies occurred in 24 hr. There was a significantly very high percentage reduction (99.9%) of viable colonies between 8 hr and 24 hr exposure time ($\rho \leq .05$). This gave total bacteria colonies elimination by the plant methanol extract.

Table 4.6: Time kill studies of methanol extracts of *Z. usamberense* at various concentration against *B. cereus* ATCC 10876

Time (hr)	CFUs±SD			CFUs±SD	% Kill or growth		
	0.5×MIC	1×MIC	2×MIC	Growth control (Extract free)	0.5×MIC	1×MIC	2×MIC
0	158.33±2.89[a]	150.67±1.54[a]	145.33±0.58[a]	158.33±2.89	NA (%)	NA (%)	NA (%)
2	74.33±4.04[b]	55.00±5.00[b]	41.67±2.89[b]	189.67±10.00	≥53.1(K)	≥63.5(K)	≥71.3(K)
4	166.00±5.29*	48.00±2.00[b]	28.00±3.21[c]	246.00±5.29	≥4.8(G)	≥68.1(K)	≥80.7(K)
6	230.00±10.00*	18.00±2.00[c]	10.33±2.89[d]	TNTC	≥45.3(G)	≥88.0(K)	≥92.9(K)
8	TNTC*	8.00±2.00[d]	5.00±2.00[e]	TNTC	TNTC(G)	≥94.5(K)	≥96.6(K)
24	TNTC*	0[e]	0[f]	TNTC	TNTC(G)	≥99.9(K)	≥99.9(K)

Descriptive statistic results were reported as Means ± SD for the triplicates. Different letters in the same column indicate significance reduction of viable colony cells. Values followed by asterix indicate the regrowth of viable colony cells

K - Kill

G - Growth

MIC - Minimum inhibitory concentration

TNTC - Too numerous to count

The results shown in Table 4.7 are the time-kill kinetics produced by stem bark methanol extract of Z. *usamberense* extracts against *Bacillus subtilis* ATCC 6633. In the growth control, colonies increased sharply from initial colonies to 242.67 ± 2.52 CFUs/ml after 4hr. Thereafter, this increased and became too numerous to count after 24 hr. In the extract concentration 0.5×MIC, there was an abrupt reduction in the bacteria colonies from original colonies to 89.33 ± 4.04 CFUs/ml after 2hr. The percentage kill of viable colonies was observed to be 45.2 % within the 2 hr exposure to plant extracts as indicated in Table 4.7. However, the colonies increased steadily to 260.67 ± 3.07 CFUs/ml after 8hr and later became too numerous to count after 24 hr. In the extracts concentration 1×MIC, there was an abrupt decline in the colonies to 9.00 ± 1.00 CFUs/ml after 6 hr exposure time. Ultimately, rapid killing of the entire colonies occurred in 24 hr. The percentage kill of viable colonies was 99.9 % at 8 hr indicating significant reduction in comparison to positive control as indicated in Table 4.7 ($\rho \leq .05$). This gave total bacteria colonies elimination by the plant methanol extracts. In the extracts concentration 2×MIC, there was a reduction of bacteria colonies from original colonies to 4.33 ± 1.53 CFUs/ml after 6hr exposure as indicated in Table 4.7. Finally, rapid killing of the entire colonies occurred in 24 hr. This was a significantly very high percentage reduction (99.9%) of viable colonies between 6 hr and 24 hr exposure ($\rho \leq 0.05$). This gave total bacteria colonies elimination by the plant methanol extracts.

Table 4.7: Time kill studies of methanol extracts of *Z. usamberense* at various concentration against *B. subtilis* ATCC 6633

Time (hr)	CFUs±SD			CFUs±SD	% Kill or growth		
	0.5×MIC	1×MIC	2×MIC	Growth control (Extract free)	0.5×MIC	1×MIC	2×MIC
0	163.00±3.60[a]	150.00±2.00[a]	148.00±2.00[a]	163.00±3.60	NA (%)	NA (%)	NA (%)
2	89.33±4.04[b]	76.67±7.64[b]	41.67±2.89[b]	169.00±3.61	≥45.2(K)	≥48.9(K)	≥71.8(K)
4	180.00±2.00*	33.67±1.53[c]	16.33±1.53[c]	242.67±2.52	≥10.4(G)	≥77.6(K)	≥89.0(K)
6	240.67±3.06*	9.00±1.00[d]	4.33±1.53[d]	TNTC	≥47.7(G)	≥94.0(K)	≥97.1(K)
8	260.67±3.07*	0[e]	0[e]	TNTC	≥59.9(G)	≥99.9(K)	≥99.9(K)
24	TNTC*	0[e]	0[e]	TNTC	TNTC(G)	≥99.9(K)	≥99.9(K)

Descriptive statistic results were reported as Means ± SD for the triplicates. Different letters in the same column indicate significance reduction of viable colony cells. Values followed by asterix indicate the regrowth of viable colony cells

K - Kill

G - Growth

MIC - Minimum inhibitory concentration

TNTC - Too numer.

The results shown in Table 4.8 are the time-kill kinetics activity produced by stem bark methanol extract of Z. *usamberense* extracts against *Staphylococcus aureus* ATCC 29213. In the growth control, colonies increased sharply from initial colonies to 244.00 ± 3.61 CFUs/ml after 4 hr. Thereafter, this increased and became too numerous to count after 24 hr. In the extracts concentration 0.5×MIC, there was a reduction in the bacteria colonies from original colonies to 100.00 ± 4.58 CFUs/ml after 4 hr interaction. The percentage kill of viable colonies was observed to be 45.1% within 4hr exposure time to plant extracts. However, the colonies increased steadily to 280.67 ± 3.05 CFUs/ml after 8 hr and became too numerous after 24 hr exposure. In the extracts concentration 1×MIC, there was a decrease in the colonies after 4 hr exposure to 96.67 ± 7.64 CFUs/ml with percentage kill of viable colonies observed to be 38.8 % in Table 4.8. This was followed by rapid growth of the colonies to 250.00 ± 2.00 CFUs/ml after 24 hr exposure to methanol extracts. In the extracts concentration 2×MIC, there was a decrease from first original colony density to 10.00 ± 2.53 CFUs/ml after 8 hr exposure. Ultimately, rapid killing of the entire colonies occurred in 24 hr after exposure to methanol extracts. The percentage kill of viable colonies was observed to be 99.9 % within the 24 hr exposure to plant methanol extracts.

Table 4.8: Time kill studies of methanol extracts of *Z. usamberense* at different concentration against *S. aureus* ATCC 29213

Time (hr)	CFUs±SD			CFUs±SD	% Kill or growth		
	0.5×MIC	1×MIC	2×MIC	Growth control (Extract free)	0.5×MIC	1×MIC	2×MIC
0	182.00±2.00[a]	158.00±2.00[a]	152.00±1.73[a]	182.00±2.00	NA (%)	NA (%)	NA (%)
2	117.67±2.52[a]	116.33±2.31[a]	115.00±5.00[a]	188.67±3.51	≥35.3(K)	≥26.4(K)	≥24.3(K)
4	100.00±4.58a	96.67±7.64[b]	65.00±5.00[b]	244.00±3.61	≥45.1(K)	≥38.8(K)	≥57.2(K)
6	200.67±2.91*	106.67±1.04*	37.67±2.52[c]	TNTC	≥10.3(G)	≥51.3(K)	≥75.2(K)
8	280.67±3.05*	225.67±4.04*	10.33±2.53[d]	TNTC	≥54.2(G)	≥42.8(G)	≥93.2(K)
24	TNTC*	250.00±2.00*	0[e]	TNTC	TNTC(G)	≥58.2(G)	≥99.9(K)

Descriptive statistic results were reported as Means ± SD for the triplicates. Different letters in the same column indicate significance reduction of viable colony cells. Values followed by asterix indicate the regrowth of viable colony cells

K - Kill

G - Growth

MIC - Minimum inhibitory concentration

TNTC - Too numerous to count

The results shown in Table 4.9 are the time-kill kinetics produced by stem bark methanol extract of *Z. usamberense* extracts against *Enterococcus faecalis* ATCC 29912. In the growth control, colonies increased sharply from initial colonies to 255.67 ± 4.04 CFUs/mL after 4 hr. Thereafter, this increased and became too numerous to count after 24 hr. In the extracts concentration 0.5×MIC, there was a 47.4 % reduction in the bacteria colonies from original colonies to 100.00 ± 2.65 CFUs/mL after 2 hr interaction. However, the colonies increased by 51.4 % steadily to 287.67 ± 1.53 CFUs/ml after 8 hr exposure and became too numerous to count after 24 hr of interaction to plant extracts. In the extracts concentration 1×MIC, there was a 41.7 % decrease in the colonies after 2 hr exposure to 98.00 ± 1.00 CFUs/mL. This was followed by 69.0 % rapid growth of the colonies to 284.00±4.00 CFUs/mL after 8 hr exposure and became too numerous to count after 24 hr. At 2×MIC extracts concentration there was a 37.6 % decrease from initial colony density to 98.67 ± 0.58 CFUs/mL after 8 hr interaction. This was followed by 10.6 % growth of the colonies to 174.67 ± 5.51 CFUs/mL after 24 hr exposure to plant methanol extracts.

Table 4.9: Time kill studies of methanol extracts of *Z. usamberense* at various concentration against *E. faecalis* ATCC 29912

Time (hr)	CFUs±SD			CFUs±SD	% Kill or growth		
	0.5×MIC	1×MIC	2×MIC	Growth control (Extract free)	0.5×MIC	1×MIC	2×MIC
0	190.00±2.00[a]	168.00±2.00[a]	158.00±2.00[a]	190.00±2.00	NA	NA	NA
2	100.00±2.65[a]	98.00±1.00[b]	88.67±0.58[b]	202.33±2.52	≥47.4(K)	≥41.7(K)	≥43.9(K)
4	142.00±1.00*	140.33±1.15*	40.67±1.15[c]	255.67±4.04	≥25.3(K)	≥16.5(K)	≥74.3(K)
6	160.00±1.00*	176.33±3.21*	68.67±0.58*	TNTC	≥15.8(K)	≥5.0(G)	≥56.5(K)
8	287.67±1.53*	284.00±4.00*	98.67±0.58*	TNTC	≥51.4(G)	≥69.0(G)	≥37.6(K)
24	TNTC*	TNTC*	174.67±5.51*	TNTC	TNTC(G)	TNTC(G)	≥10.6(G)

Descriptive statistic results were reported as Means ± SD for the triplicates. Different letters in the same column indicate significance reduction of viable colony cells. Values followed by asterix indicate the regrowth of viable colony cells

K - Kill

G - Growth

MIC - Minimum inhibitory concentration

TNTC - Too numerous to count

The results shown in Table 4.10 are time-kill kinetics produced by stem bark methanol extract of *Z. usamberense* against *Escherichia coli* ATCC25922. In the growth control, colonies increased steadily from initial colonies to 281.00 ± 1.53 CFUs/mL after 2 hr. Thereafter, this increased and became too numerous to count after 24 hr interaction. In the extracts concentration 0.5×MIC, there was a decline in the bacteria colonies from original colony density to 103.00 ± 2.89 CFUs/mL after 4 hr. However, the colonies increased steadily by 22 % to 195.00 ± 5.00 CFUs/mL after 6 hr and became too numerous after 24 hr exposure time with plant extracts. In the extracts concentration 1×MIC, there was a decrease in the colonies after 4 hr exposure to 96.67 ± 7.64 CFUs/mL. This was followed by 9.5 % growth of the colonies to 180.67 ± 5.00 after 6 hr exposure and later became too numerous to count after 24 hr with plant extracts. In the extracts concentration 2×MIC, there was a 69.5 % decrease from initial colony density to 55.00 ± 5.00 CFUs/ml after 6hr exposure time. This was followed by 47.5 % increase of the colonies up to 266.00 ± 7.67 CFUs/mL in 24hr exposure time with plant extracts.

Table 4.10: Time kill studies of methanol extracts of *Z. usamberense* at various concentration against *E. coli* ATCC 25922

Time (hr)	CFUs±SD			CFUs±SD	% Kill or growth		
	0.5×MIC	1×MIC	2×MIC	Growth control (Extract free)	0.5×MIC	1×MIC	2×MIC
0	250.00±1.00[a]	199.00±1.00[a]	180.33±1.53[a]	250.00±1.53	NA	NA	NA
2	131.67±7.63[a]	111.67±7.64[a]	101.67±7.64[a]	281.67±1.53	≥47.3(K)	≥43.9(K)	≥43.6(K)
4	103.33±2.89[a]	96.67±7.64[b]	95.67±4.04[b]	TNTC	≥58.7(K)	≥51.4(K)	≥46.9(K)
6	195.00±5.00[*]	180.00±5.00[*]	55.00±5.00[c]	TNTC	≥22.0(G)	≥9.5(G)	≥69.5(K)
8	285.00±4.58[*]	284.67±5.03[*]	157.33±2.51[*]	TNTC	≥14.0(G)	≥43.1(G)	≥12.8(K)
24	TNTC[*]	TNTC[*]	266.00±7.67[*]	TNTC	TNTC(G)	TNTC(G)	≥47.5(G)

Descriptive statistic results were reported as Means ± SD for the triplicates. Different letters in the same column indicate significance reduction of viable colony cells. Values followed by asterix indicate the regrowth of viable colony cells

K - Kill

G - Growth

MIC - Minimum inhibitory concentration

TNTC - Too numerous to count

4.7 *In vivo* toxicity studies

4.7.1 The general signs, behavior and mortality analysis of mice

The Swiss albino mice were monitored in both control group and extract-treated groups in both routes. The first week of extracts treated-groups, mice showed reduction of feed intake which later became normal for the rest of the weeks in oral routes. The animal showed no changes in stool, urine and eye color of extracts treated animals. They did not display changes in behavior, posture and hair loss. There was no mortality in the extract treated mice in both routes. The other clinical toxicity signs observed include drowsiness, muscle twisting and restlessness but there were no manifestations.

4.7.2 Extracts toxicity

4.7.2.1 Effect of methanol plant extracts on body weight in mice

The data in Tables 4.11 and 4.12 indicate the toxic effect of varying doses of *Z. usamberense* methanol extract given orally and intraperitoneally in male Swiss albino mice after 4 weeks on body weights. There were changes in the body weight increase in mice for the whole administered groups. The administration of extract to the animals after 4 weeks gave a drug dependent ($\rho \leq 0.05$) weight reduction as herbal drug increased in both routes. Dosing of 1000 mg/kg body weight indicated the lowest weight increase compared to control group in oral routes. Despite this, herbal drug indicated significant ($\rho \leq 0.05$) increase in body weight after the first week in both routes as observed from weight values. Intraperitoneally administered herbal drug indicated variable changes in weight of mice given 1000 mg/kg body weight during the second, third and fourth week. In control group there was gradual weight gain throughout the dosing period of treatment in both routes.

Table 4.11: Effect of *Z. usamberense* methanol extract given orally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Body weight					Δ grams/week
	0	**1**	**2**	**3**	**4**	
Control	23.60±1.14^{b}	25.50±1.07^{b}	27.32±1.08^{b}	29.08±0.87^{b}	30.88±0.84^{c}	1.82±0.09^{c}
450	19.26±2.21^{b}	21.28±2.42^{ab}	23.22±2.37^{b}	25.12±2.32^{b}	26.76±2.09^{bc}	1.88±0.05^{c}
670	17.68±3.96^{a}	19.78±3.70^{a}	24.06±3.57^{b}	25.28±3.48^{b}	26.40±3.41^{b}	1.14±0.12^{b}
1000	16.36±2.66^{a}	17.52±2.26^{a}	18.36±2.27^{a}	19.20±2.24^{a}	19.88±2.11^{a}	0.88±0.15^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key; Δ-shows weight changes

Table 4.12: Effect of *Z. usamberense* methanol extract given intraperitoneally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Body weight					Δ grams/week
	0	**1**	**2**	**3**	**4**	
Control	23.20±1.48^{b}	25.20±1.89^{c}	27.14±1.80^{b}	28.98±1.80^{b}	30.72±1.81^{b}	1.88±0.13^{b}
450	16.82±1.27^{a}	17.58±3.70^{b}	20.30±1.29^{a}	21.90±1.51^{a}	23.46±1.33^{a}	2.10±0.53^{b}
670	18.14±1.87^{a}	18.22±1.76^{a}	20.38±1.90^{a}	21.36±1.71^{a}	22.50±1.78^{a}	1.09±0.04^{a}
1000	21.63±1.97^{a}	19.20±1.90^{a}	21.28±1.90^{a}	23.54±1.82^{a}	24.20±1.99^{a}	0.71±0.09^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key: Δ-shows weight changes

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The results in Tables 4.13 and 4.14 indicate the toxic consequences of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally in mice after 4 weeks on body weights. For the entire dosing period, there were changes in the body weight gain of mice in all the treated groups in both routes. From the Tables, the administration of methanol extracts indicated dose dependent reduction in weight gain as herbal drug increased across the treated groups ($\rho \leq 0.05$). In spite of this, oral dosage of 450 mg/kg body weight as per weight of body showed considerable increase in body weight as from second week and intraperitoneal picking from fourth week as observed from values ($\rho \leq 0.05$). However, the dosing of herbal drug showed variable changes in weight gain in Swiss albino mice administered with 670 and 1000 mg/kg body weight for the entire dosing period for both routes. Animals in control set up gained weight throughout the dosing period of treatment in all routes.

Table 4.13: Effect of *Z. chalybeum* methanol extract given orally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	**Body weight** 0	1	2	3	4	**Δ grams/week**
Control	23.60±1.14[a]	25.50±1.07[a]	27.32±1.08[b]	29.08±0.87[b]	30.88±0.84[b]	1.82±0.09[b]
450	23.60±1.14[a]	25.50±1.08[a]	26.50±1.06[ab]	28.22±0.93[b]	29.40±0.90[b]	1.47±0.10[ab]
670	23.20±1.48[a]	24.28±1.38[a]	25.12±1.43[ab]	26.22±1.40[a]	27.28±1.38[a]	1.02±0.09[a]
1000	23.40±1.44[a]	24.90±1.94[a]	24.82±1.10[a]	25.50±1.24[a]	26.04±1.23[a]	0.66±0.06[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key: Δ-shows weight changes

Table 4.14: Effect of *Z. chalybeum* methanol extract given intraperitoneally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	**Body weight** 0	1	2	3	4	**Δ grams/week**
Control	23.20±1.48[a]	25.20±1.89[a]	27.14±1.80[a]	28.98±1.80[a]	30.72±1.81[b]	1.88±0.13[b]
450	23.40±1.34[a]	24.92±1.21[a]	26.26±1.33a	27.52±1.21[a]	28.80±1.20[ab]	1.35±0.07[ab]
670	23.00±1.58[a]	24.00±1.73[a]	24.94±1.71[a]	25.96±1.71[a]	26.94±1.72[a]	0.99±0.06[a]
1000	23.60±1.67[a]	24.66±1.56[a]	25.24±1.52[a]	25.80±1.51[a]	26.58±1.82[a]	0.75±0.15[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key: Δ-shows weight changes

The results in Table 4.15 and 4.16 indicate the toxic consequences of varying doses of *Aloe deserti* methanol extract given orally and intraperitoneally in mice after 4 weeks on body weights. For entire dosing period, there were changes in the body weight gain of mice in all the treated groups in both routes. As indicated from the Table 4.15 and 4.16, the administration of methanol leaf extract of *Aloe deserti* after 4 weeks gave a reduction in weight gain as the dose increased across all the treated groups ($\rho \leq 0.05$). In spite of this, the dosage of 450 and 670 mg/kg body weight of herbal drug showed considerable decrease in body weight that started from second week in oral administration and third week in intraperitoneal administration as observed from values ($\rho \leq 0.05$). However, the dosing of herbal drug showed variable changes in weight gain of Swiss albino mice given 1000 mg/kg body weight for entire dosing period in both routes. Animals in control group increased their weight throughout the dosing period.

Table 4.15: Effect of *A. deserti* methanol extract given orally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Body weight 0	1	2	3	4	Δ grams/week
Control	23.60±1.14^{a}	25.50±1.07^{a}	27.32±1.08^{b}	29.08±0.87^{b}	30.88±0.84^{b}	1.82±0.09^{c}
450	23.60±1.14^{a}	24.84±1.19^{a}	26.02±1.23^{ab}	27.18±1.19^{a}	28.42±1.16^{a}	1.21±0.04^{b}
670	23.40±1.14^{a}	24.42±1.19^{a}	25.54±1.17^{ab}	26.40±1.25^{a}	27.36±1.42^{a}	0.99±0.20^{b}
1000	23.00±1.00^{a}	24.64±1.08^{a}	24.94±0.89^{a}	26.10±0.81^{a}	26.60±0.78^{a}	0.65±0.10^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key: **Δ-shows weight changes**

Table 4.16: Effect of *A. deserti* methanol extract given intraperitoneally on body weights of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Body weight 0	1	2	3	4	Δ grams/week
Control	23.20±1.48^{a}	25.20±1.89^{a}	27.14±1.80^{a}	28.98±1.80^{b}	30.72±1.81^{c}	1.88±0.13^{d}
450	23.80±1.30^{a}	25.16±1.26^{a}	26.38±1.26^{a}	27.66±1.32^{ab}	29.06±1.38^{c}	1.32±0.03^{c}
670	23.60±1.40^{a}	24.56±1.07^{a}	25.42±1.13^{a}	26.26±1.22^{a}	27.12±1.24^{ab}	0.88±0.07^{b}
1000	22.80±1.58^{a}	24.42±1.47^{a}	24.94±1.51^{a}	25.54±1.58^{a}	26.10±1.59^{a}	0.53±0.07^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.
Key: **Δ-shows weight changes**

4.7.2.2 Effect of methanol plant extracts on organ weight in mice

The results in Table 4.17 and 4.18 indicate the toxic effect of varying doses of Z. *usamberense* methanol extract given orally and intraperitoneally after 4 weeks on weight of organs of Swiss albino mice. As indicated from the Tables, the administration of varying extract doses displayed a considerable decrease in weights of brain and kidneys compared to the control groups in both routes ($\rho \leq 0.05$). As shown im the Table Table 4.17 and 4.18, there were variable changes in weight of liver, testes and heart compared to control group in all routes. Intraperitoneal dosing of herbal extract indicated remarkable changes in percent organ to body weight of lungs, spleen and kidney in the treated groups relative to control group ($\rho \leq 0.05$). Intraperitoneal dosing of 670 mg/kg body weight indicated decline in percentage organ to body weight of spleen and brain compared to control group ($\rho \leq 0.05$). In oral administration, the percent organ to body weight of spleen and kidney indicated variable changes. Oral dosing indicated remarkable percent organ to body weight changes of brain and lungs with the lowest weight gain being at 450 mg/kg body weight when compared to control group.

Table 4.17: Effect of *Z. usamberensse* methanol extract given orally on organ weight of Swiss albino mice after 4 weeks

Treatment	Organ weights (g)						
(mg/kgbw)	Spleen	Testes	Kidneys	Brain	Lungs	Heat	Liver
Control	0.31±0.01[a]	0.30±0.03[a]	0.61±0.01[b]	0.82±0.01[b]	0.41±0.01[b]	0.14±0.01[a]	1.63±0.02[a]
450	0.24±0.03[a]	0.28±0.04[a]	0.49±0.04[a]	0.68±0.03[ab]	0.29±0.10[a]	0.14±0.03[a]	1.78±0.11[a]
670	0.22±0.07[a]	0.36±0.11[a]	0.51±0.09[a]	0.69±0.11[ab]	0.38±0.06[ab]	0.16±0.02[a]	1.80±0.23[a]
1000	0.24±0.09[a]	0.31±0.06[a]	0.43±0.06[a]	0.62±0.17[a]	0.34±0.06[ab]	0.18±0.08[a]	1.92±0.28[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.18: Effect of *Z. usamberense* methanol extract given intraperitoneally on organ weight of Swiss albino mice after 4 weeks

Treatment	Organ weights (g)						
(mg/kgbw)	Spleen	Testes	Kidneys	Brain	Lungs	Heart	Liver
Control	0.31±0.02[c]	0.30±0.03[a]	0.61±0.01[c]	0.81±0.01[c]	0.41±0.01[b]	0.15±0.01[a]	1.62±0.02[a]
450	0.24±0.03[bc]	0..28±0.04[a]	0.49±0.04[b]	0.69±0.03[b]	0.29±0.10[ab]	0.15±0.10[a]	1.78±0.11[a]
670	0.17±0.03[a]	0.31±0.04[a]	0.51±0.07[b]	0.66±0.07[b]	0.29±0.02[a]	0.14±0.02[a]	1.74±0.31[a]
1000	0.21±0.06[ab]	0.25±0.08[a]	0.40±0.05[a]	0.47±0.07[a]	1.24±0.10[a]	0.13±0.03[a]	1.75±0.13[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

The results in Tables 4.19 and 4.20 indicate the effect of *Z. chalybeum* methanol extract given orally and intraperitoneally after 4 weeks on weight of organs in Swiss albino mice. As depicted from the tables, the administration of the extracts showed remarkable changes in organ weight of spleen, kidney, lungs brain and heart during the entire treatment period compared to control mice in both routes ($\rho \leq 0.05$). Oral dosing extract to animals indicated significant changes of liver weight gain compared to control groups ($\rho \leq 0.05$). Intraperitoneal dosing of herbal drugs showed unaffected weight gain of liver relative to control group. There was a significant decrease in testes weight gain during intraperitoneal administration of herbal drug relative to control group ($\rho \leq 0.05$). The decrease of testes weights in the oral route was not statistically significant.

Table 4.19: Effect of *Z. chalybeum* methanol extract given orally on organ weight of Swiss albino mice after 4 weeks

Treatment	Organ weights (g)						
(mg/kgbw)	Spleen	Liver	Kidneys	Brain	Testes	Heart	Lungs
Control	$0.32{\pm}0.02^{b}$	$1.61{\pm}0.02^{ab}$	$0.61{\pm}0.01^{b}$	$0.78{\pm}0.06^{b}$	$0.30{\pm}0.03^{a}$	$0.15{\pm}0.01^{b}$	$0.41{\pm}0.01^{b}$
450	$0.24{\pm}0.01^{a}$	$1.65{\pm}0.01^{b}$	$0.52{\pm}0.01^{a}$	$0.73{\pm}0.01^{ab}$	$0.29{\pm}0.02^{a}$	$0.13{\pm}0.01^{a}$	$0.31{\pm}0.01^{a}$
670	$0.23{\pm}0.02^{a}$	$1.63{\pm}0.02^{b}$	$0.50{\pm}0.01^{a}$	$0.73{\pm}0.02^{ab}$	$0.26{\pm}0.04^{a}$	$0.13{\pm}0.01^{a}$	$0.29{\pm}0.02^{a}$
1000	$0.26{\pm}0.02^{a}$	$1.60{\pm}0.03^{a}$	$0.50{\pm}0.02^{a}$	$0.69{\pm}0.02^{a}$	$0.26{\pm}0.04^{a}$	$0.13{\pm}0.01^{a}$	$0.29{\pm}0.02^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.20: Effect of *Z. chalybeum* methanol extract given intraperitoneally on organ weight of Swiss albino mice after 4 weeks

Treatment	Organ weights (g)						
(mg/kgbw)	Spleen	Testes	Kidneys	Lungs	Heart	Liver	Bain
Control	$0.31{\pm}0.02^{b}$	$0.30{\pm}0.02^{b}$	$0.61{\pm}0.01^{b}$	$0.42{\pm}0.01^{b}$	$0.14{\pm}0.01^{b}$	$1.61{\pm}0.01^{ab}$	$0.80{\pm}0.02^{b}$
450	$0.23{\pm}0.01^{a}$	$0.28{\pm}0.01^{ab}$	$0.50{\pm}0.01^{a}$	$0.31{\pm}0.01^{a}$	$0.13{\pm}0.01^{a}$	$1.64{\pm}0.01^{b}$	$0.73{\pm}0.02^{a}$
670	$0.25{\pm}0.02^{a}$	$0.27{\pm}0.03^{ab}$	$0.50{\pm}0.02^{a}$	$0.28{\pm}0.01^{a}$	$0.13{\pm}0.01^{a}$	$1.62{\pm}0.02^{ab}$	$0.71{\pm}0.04^{a}$
1000	$0.26{\pm}0.02^{a}$	$0.26{\pm}0.01^{a}$	$0.50{\pm}0.02^{a}$	$0.29{\pm}0.03^{a}$	$0.13{\pm}0.01^{a}$	$1.60{\pm}0.03^{a}$	$0.69{\pm}0.02^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

The results in Table 4.21 and 4.22 indicate the toxic effect of *Aloe deserti* methanol extract given orally and intraperitoneally to Swiss albino mice after 4 weeks. As indicated in Table 4.21 and 4.22, the administration of varying doses of herbal drug gave significant decrease in weights especially for brain, lungs and testes in comparison to the control groups in both routes ($\rho \leq 0.05$). Oral dispensation of the herbal drug indicated significant dosage dependent decrease in weight of liver and heart compared to control groups ($\rho \leq 0.05$). However, intraperitoneal dosing indicated significant changes in the weight of liver and heart compared to control groups. Oral dosing indicated remarkable weight changes of kidneys and spleen compared to control group ($\rho \leq 0.05$). Intraperitoneal dosing indicated significant dose dependent decrease in kidneys and liver weight compared to control groups ($\rho \leq 0.05$).

Table 4.21: Effect of *A. deserti* methanol extract given orally on organ weight of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Organ weights (g) Spleen	Testes	Kidneys	Brain	Lungs	Heart	Liver
Control	$0.31{\pm}0.01^{b}$	$0.30{\pm}0.03^{c}$	$0.61{\pm}0.01^{b}$	$0.82{\pm}0.01^{c}$	$0.41{\pm}0.01^{c}$	$0.14{\pm}0.01^{c}$	$1.63{\pm}0.02^{c}$
450	$0.29{\pm}0.01^{ab}$	$0.27{\pm}0.02^{bc}$	$0.58{\pm}0.04^{b}$	$0.78{\pm}0.03^{bc}$	$0.67{\pm}0.02^{b}$	$0.13{\pm}0.01^{bc}$	$1.61{\pm}0.01^{bc}$
670	$0.28{\pm}0.04^{ab}$	$0.23{\pm}0.02^{ab}$	$0.53{\pm}0.02^{a}$	$0.74{\pm}0.05^{ab}$	$0.35{\pm}0.02^{ab}$	$0.13{\pm}0.01^{ab}$	$1.57{\pm}0.04^{ab}$
1000	$0.25{\pm}0.01^{a}$	$0.23{\pm}0.01^{a}$	$0.53{\pm}0.02^{a}$	$0.71{\pm}0.01^{a}$	$0.33{\pm}0.01^{a}$	$0.12{\pm}0.01^{a}$	$1.52{\pm}0.02^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.22: Effect of *A. deserti* methanol extract given intraperitoneally on organ weight of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Organ weights (g) Spleen	Testes	Kidneys	Brain	Lungs	Heart	Liver
Control	$0.31{\pm}0.02^{b}$	$0.30{\pm}0.03^{c}$	$0.61{\pm}0.01^{c}$	$0.81{\pm}0.01^{c}$	$0.41{\pm}0.01^{c}$	$0.15{\pm}0.01^{b}$	$1.62{\pm}0.02^{b}$
450	$0.29{\pm}0.03^{b}$	$0.27{\pm}0.01^{bc}$	$0.56{\pm}0.05^{bc}$	$0.78{\pm}0.03^{bc}$	$0.38{\pm}0.02^{b}$	$0.14{\pm}0.01^{b}$	$1.61{\pm}0.01^{b}$
670	$0.28{\pm}0.04^{b}$	$0.23{\pm}0.02^{ab}$	$0.55{\pm}0.02^{ab}$	$0.74{\pm}0.03^{ab}$	$0.35{\pm}0.02^{ab}$	$0.12{\pm}0.01^{a}$	$1.57{\pm}0.04^{a}$
1000	$0.24{\pm}0.02^{a}$	$0.23{\pm}0.02^{a}$	$0.52{\pm}0.04^{a}$	$0.89{\pm}0.06^{a}$	$0.32{\pm}0.02^{a}$	$0.11{\pm}0.01^{a}$	$1.52{\pm}0.02^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

4.7.2.3 Percentage of organ to body weight of mice

The data in Table 4.23 and 4.24 indicate the effect of varying dosage of *Z. usamberense* methanol extract given orally and intraperitoneally after 4 weeks on relative percentage of organ to weight of body. As indicated in Table 4.23 and 4.24, the herbal drug indicated a dose dependent elevation in relative percentage of heart weight to body weight compared to that of control group in both routes ($\rho \leq 0.05$). The weight changes of spleen and testes in both administrations were not statistically significant including that of lungs in intraperitoneal administration compared to control groups. Oral doses gave unaffected percentage of organ to body weight of lungs compared to control group. Oral dosing indicated no significant changes in liver, kidneys and brain relative to control groups. As indicated from Table 4.23 and 4.24, intraperitoneal administration gave significant percentage weight decrease in brain and kidney and significant elevation in percentage weight of liver relative to control groups ($\rho \leq 0.05$).

Table 4.23: Effect of *Z. usamberense* methanol extract given orally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent of organ to weight of body						
(mg/kg bw)	Spleen	Liver	Kidneys	Brain	Lungs	Heart	Testes
Control	1.00±0.04[a]	5.28±0.11[a]	1.98±0.06[a]	2.64±0.06[a]	1.34±0.02[ab]	0.47±0.13[a]	0.98±0.79[a]
450	0.92±0.14[a]	6.69±0.86[a]	1.86±0.25[a]	2.61±0.74[a]	1.09±0.44[a]	0.52±0.14[a]	1.06±0.22[a]
670	0.90±0.40[a]	6.98±1.65[a]	1.95±0.48[a]	2.68±0.78[a]	1.46±0.14[ab]	0.63±0.13[ab]	1.42±0.57[a]
1000	1.25±0.50[a]	9.64±0.86[b]	2.21±0.43[a]	3.10±0.74[a]	1.77±0.40[b]	0.87±0.28[b]	1.59±0.34[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.24: Effect of *Z. usamberense* methanol extract given intraperitoneally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent of organ to weight of body						
(mg/kgbw)	Spleen	Liver	Kidneys	Brain	Lungs	Heart	Testes
Control	1.02±0.07[a]	5.30±0.26[a]	2.00±0.12[ab]	2.64±0.15[b]	1.35±0.06[a]	0.48±0.05[a]	0.99±0.07[a]
450	1.06±0.20[a]	7.85±0.85[b]	2.59±0.84[b]	3.12±0.29[b]	1.35±0.38[a]	0.48±0.05[a]	1.21±0.33[a]
670	0.75±0.13[a]	7.69±0.84[b]	2.28±0.30[ab]	2.95±0.29[a]	1.29±1.63[a]	0.66±0.10[b]	1.38±0.21[a]
1000	0.87±0.30[a]	7.29±1.00[b]	1.65±0.25[a]	1.96±0.35[a]	0.97±0.40[a]	0.54±0.13[b]	1.03±0.35[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

The data in Table 4.25 and 4.26 indicate the effect of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally after 4 weeks on percentage organ to body weight of mice. As depicted in Table 4.25 and 4.26, no statistical changes in percentage organ to body weight of heart and testes compared to control groups in both routes. However, the herbal drug gave a dose dependent elevation in the relative percentage organs weight of liver weight to body weight compared to that of control experimental mice in both routes ($\rho \leq 0.05$). As indicated in Table 4.25 and 4.26, there were variable changes in the relative percentage organs weight of kidney and spleen with the lowest percentage organ to body weight being at 450 mg/kg body weight compared to control groups in both routes. In oral dosing, relative percentage organ weight to body weight of brain indicated unaltered changes compared to control group. Intraperitoneal administration resulted in no significant statistical percent organ to body weight changes in brain compared to control group. The changes in the relative percentage organs weight of lungs to body weight declined as dose increased in both routes.

Table 4.25: Effect of *Z. chalybeum* methanol extract given orally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent of organ to weight of body						
(mg/kgbw)	Spleen	Liver	Kidneys	Brain	Lungs	Heart	Testes
Control	1.06±0.08^{c}	5.33±0.22^{a}	2.00±0.05^{c}	1.06±0.08^{c}	1.35±0.07^{b}	0.48±0.02^{a}	0.99±0.07^{a}
450	0.81±0.04^{a}	5.59±0.16^{ab}	1.75±0.07^{a}	0.81±0.04^{a}	1.07±0.05^{a}	0.45±0.02^{a}	1.00±0.08^{a}
670	0.85±0.08^{ab}	6.00±0.25^{bc}	1.85±0.07^{ab}	0.85±0.08^{ab}	1.07±0.05^{a}	0.46±0.03^{a}	0.95±0.13^{a}
1000	0.99±0.10^{bc}	6.14±0.29^{c}	1.92±0.11^{bc}	0.99±0.10^{bc}	1.13±0.13^{a}	0.48±0.04^{a}	1.02±0.20^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.26: Effect of *Z. chalybeum* methanol extract given intraperitoneally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent of organ to weight of body						
(mg/kgbw)	Spleen	Liver	Kidneys	Brain	Lungs	Heart	Testes
Control	1.00±0.07^{b}	5.18±0.18^{a}	1.95±0.04^{b}	2.58±0.07^{a}	1.34±0.04^{b}	0.46±0.02^{a}	0.97±0.05^{a}
450	0.80±0.04^{a}	5.69±0.22^{b}	1.74±0.07^{a}	2.54±0.04^{a}	1.08±0.02^{a}	0.44±0.02^{a}	0.96±0.06^{a}
670	0.94±0.05^{b}	6.04±0.31^{b}	1.85±0.06^{ab}	2.65±0.07^{a}	1.05±0.07^{a}	0.47±0.02^{a}	1.02±0.16^{a}
1000	0.97±0.05^{b}	6.03±0.34^{b}	1.88±0.09^{b}	2.62±0.09^{a}	1.11±0.05^{a}	0.48±0.02^{a}	0.99±0.08^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

The data in Table 4.27 and 4.28 indicate the effect of varying doses of *Aloe deserti* methanol extract given orally and intraperitoneally after 4 weeks on percentage organs to body weight of mice. As indicated in Table 4.27 and 4.28, there were variable changes in percentage organ to to body weight of the heart, brain, kidney and spleen of treated mice in all routes. Despite this, herbal drug gave a dose dependent elevation of percent organ to body weight of the liver in both routes ($\rho \leq .05$). Surprisingly, plant extracts caused statistically significant decrease in percentage organ to body weight of testes in both routes ($\rho \leq .05$). Oral dosing of the herbal extracts indicated variable changes in percent organ to body weight of lungs during entire treatment period. However, intraperitoneal dosing of herbal drug caused significant reduction in percent weight gain of lungs compared to control groups ($\rho \leq 0.05$).

Table 4.27: Effect of *A. deserti* methanol extract given orally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent organ to body weight						
(mg/kgbw)	Spleen	Testes	Kidneys	Brain	Lungs	Heart	Liver
Control	1.00±0.04[a]	0.98±0.07[b]	2.07±1.98[a]	2.64±0.06[a]	1.35±0.02[a]	0.47±0.01[a]	5.29±0.11[a]
450	1.01±0.04[a]	0.94±0.21[ab]	2.04±0.13[a]	2.74±0.08[a]	1.29±0.10[a]	0.47±0.02[a]	5.66±0.21[b]
670	1.01±0.09[a]	0.85±0.04[a]	1.95±0.04[a]	2.70±0.06[a]	1.29±0.05[a]	0.46±0.03[a]	5.78±0.20[b]
1000	0.09±0.02[a]	0.84±0.03[a]	1.97±0.07[a]	2.68±0.05[a]	1.25±0.03[a]	0.44±0.02[a]	5.79±0.13[b]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

Table 4.28: Effect of *A. deserti* methanol extract given intraperitoneally on percentage organ to body weight of Swiss albino mice after 4 weeks

Treatment	Relative percent of organ to weight of body						
(mg/kgbw)	Spleen	Testes	Kidneys	Brain	Lungs	Heart	Liver
Control	1.02±0.07[a]	0.99±0.07[b]	2.00±0.12[a]	2.64±0.15[a]	1.35±0.06[b]	0.48±0.05[a]	5.30±0.26[a]
450	1.00±0.07[a]	0.92±0.02[ab]	2.02±0.10[a]	2.69±0.09[a]	1.29±0.04[ab]	0.45±0.02[a]	5.56±0.29[ab]
670	1.04±0.10[a]	0.87±0.09[ab]	2.01±0.06[a]	2.72±0.09[a]	1.30±0.04[ab]	0.46±0.02[a]	5.78±0.15[b]
1000	0.93±0.12[a]	0.86±0.08[a]	1.98±0.05[a]	2.61±0.10[a]	1.24±0.07[a]	0.46±0.03[a]	5.85±0.32[b]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant.

4.8 Hematological parameters

4.8.1 Analysis of erythrocytes and related parameters

The data in Table 4.29 and 4.30 indicate the effect of varying doses of *Z. usamberense* methanol extract given orally and intraperitoneally in Swiss albino mice after 4 weeks on red blood cells, hemoglobin and the related parameters. As depicted from the two tables, the `dosage of plant extracts both orally and intraperitoneally to mice after 4 weeks at all dose levels increased RBC, Hb, PCV and MCH across entire treatments and showed statistically significant difference compared control groups ($\rho \leq 0.05$). Similarly, continued daily administration of plant extract gave statistically significant elevation in RBC and MCH that started at 450 mg/kg body weight in both routes ($\rho \leq 0.05$). A decrease in MCHC values was observed across the four treatments. Oral dosing of plant extracts indicated statistically significant elevation of MCV as drug concentration increased relative to control groups ($\rho \leq 0.05$). The intraperitoneally administratered plant extract reveals variable changes in MCV values compared to control groups. Oral and intraperitoneal administration showed significant increase in RDW begining at 450 mg/kg body weight and oral dosing gave significant increase compared to control group ($\rho \leq 0.05$). Orally and intraperitoneally administratered herbal drug indicated a dose dependent ($\rho \leq 0.05$) reduction in glucose levels in comparison with control groups.

Table 4.29: Effect of *Z. usamberense* methanol extract given orally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment	Erythrocytes and accessible parameters							
(mg/kgbw)	RBC($x10^{12}$/L)	Hb(g/dL)	PCV (pg)	MCH(pg)	MCHC(g/dL)	MCV(fL)	RDW (%)	GLU(mmol/L)
Control	5.60±0.54^{a}	7.98±1.73^{a}	22.36±0.89^{a}	14.27±2.72^{a}	35.96±9.42^{a}	40.33±5.29^{a}	18.70±1.47^{a}	5.64±1.20^{b}
450	6.76±1.04^{ab}	11.60±1.14^{b}	36.20±2.39^{b}	17.33±1.90^{ab}	32.27±4.84^{a}	54.89±11.02^{bc}	19.24±0.47^{ab}	4.98±0.47^{b}
670	7.60±1.14^{bc}	14.40±0.54^{b}	40.00±1.58^{c}	19.25±2.61^{b}	30.02±1.28^{a}	64.25±9.55^{cd}	20.62±0.79^{b}	3.56±0.56^{a}
1000	9.18±0.81^{c}	19.60±2.70^{c}	71.80±2.68^{d}	21.43±3.04^{b}	27.25±3.11^{a}	78.60±6.05^{d}	24.10±0.55^{c}	2.66±0.42^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

Table 4.30: Effect of *Z. usamberense* methanol extract given intraperitoneally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment	Erythrocytes and accessible parameters							
(mg/kg bw)	RBC ($x10^{12}$/L)	Hb (g/dL)	PCV (pg)	MCH (pg)	MCHC(g/dL)	MCV (fL)	RDW (%)	GLU(mmol/L)
Control	6.06±0.37^{a}	8.98±1.33^{a}	26.53±0.95^{a}	14.93±3.92^{a}	33.85±6.49^{a}	43.93±3.24^{a}	20.20±1.10^{a}	5.38±0.36^{c}
450	7.60±0.54^{ab}	12.40±1.52^{b}	41.20±1.92^{b}	16.29±1.25^{b}	30.11±3.71^{a}	54.46±5.15^{a}	23.50±2.57^{a}	4.82±0.35^{bc}
670	8.60±1.52^{bc}	14.00±0.71^{b}	49.40±1.67^{c}	16.62±2.52^{b}	28.38±2.04^{a}	58.75±9.57^{a}	25.34±4.88^{ab}	4.14±0.60^{b}
1000	9.80±1.64^{c}	17.40±1.82^{c}	63.00±3.67^{d}	18.05±2.79^{b}	27.66±3.02^{a}	65.48±9.94^{a}	30.10±2.13^{b}	3.58±0.38^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

The data in Table 4.31 and 4.32 indicate the effect of varying doses of *Z. chalybeum* methanol extracts given orally and intraperitoneally in Swiss albino mice after 4 weeks on red blood cells, hemoglobin and the related indices and glucose levels. As depicted from the tables, oral and intraperitoneally administered plant extract increased the levels of RBC, Hb, PCV and MCV throughout the entire treatments and were statistically ($\rho \leq 0.05$) significant from control groups. Similarly, continued dosing of extract daily caused a significant ($\rho < 0.05$) increase in RBC starting at 450 mg/kg body weight as shown in all treatment of both routes. Dose dependent ($\rho \leq 0.05$) increase in MCV started at 450 mg/kg body weight in both routes. Dosing plant extract orally revealed significant increase in MCH levels as the dose increased compared to control groups. Intraperitoneal dosing of plant extracts revealed variable changes in MCH levels as the dose increased compared to control groups. As shown from the tables, oral administration caused a significant decrease in MCHC levels relative to control groups. However, intraperitoneal administration revealed statistically significant ($\rho \leq 0.05$) decrease in MCHC levels as the dose of the extract increased compared to control groups. Oral and intraperitoneal administration showed significant ($\rho \leq 0.05$) increase in RDW value that picked at 450 mg/kg body weight compared to controls. Orally and intraperitoneally administratered herbal drug indicated a dose dependent ($\rho \leq 0.05$) reduction in glucose levels in comparison with control groups.

Table 4.31: Effect of *Z. chalybeum* methanol extract given orally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Erythrocytes and accessible parameters							
	RBC (x10^{12}/L)	Hb (g/dL)	PCV (pg)	MCH (pg)	MCHC (%)	MCV (fL)	RDW (%)	GLU (mmol/L)
Control	5.60±0.54^{a}	7.98±1.73^{a}	22.36±0.89^{a}	14.27±2.72^{a}	35.96±9.42^{a}	40.33±5.29^{a}	18.70±1.47^{a}	5.64±1.20^{b}
450	7.06±0.85^{ab}	11.60±1.14^{b}	34.80±1.64^{b}	16.52±8.04^{ab}	33.44±4.11^{a}	50.04±8.04^{ab}	20.46±1.13^{ab}	4.34±0.47^{ab}
670	8.36±1.07^{bc}	14.20±0.84^{c}	48.80±3.19^{c}	17.17±2.09^{ab}	30.44±2.55^{a}	56.57±6.71^{b}	21.60±2.07^{b}	3.49±0.76^{a}
1000	9.12±0.76^{c}	16.60±1.14^{d}	59.40±2.70^{d}	18.33±2.23^{c}	28.02±2.63^{a}	65.37±4.17^{c}	23.00±1.58^{b}	3.26±0.28^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

Table 4.32: Effect of *Z. chalybeum* methanol extract given intraperitoneally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Erythrocytes and accessible parameters							
	RBC (x10^{12}/L)	Hb (g/dL)	PCV (pg)	MCH (pg)	MCHC (g/dL)	MCV (fL)	RDW (%)	GLU(mmol/L)
Control	6.06±0.37^{a}	8.98±1.33^{a}	26.53±0.95^{a}	14.91±2.68^{a}	33.85±5.65^{b}	43.93±3.24^{a}	20.20±1.10^{a}	5.38±0.36^{c}
450	7.36±1.06^{ab}	11.20±1.48^{a}	37.80±1.64^{b}	15.50±3.15^{a}	29.75±4.89^{ab}	52.34±8.78^{ab}	23.20±1.48^{b}	4.24±0.23^{b}
670	8.06±0.33^{bc}	13.60±0.89^{b}	49.40±2.07^{c}	16.90±1.36^{a}	27.51±0.86^{ab}	61.39±3.93^{bc}	24.20±1.30^{b}	3.61±0.58^{ab}
1000	9.18±1.29^{c}	15.80±1.48^{b}	59.20±1.92^{d}	17.39±2.14^{a}	26.70±2.54^{a}	65.28±7.24^{c}	25.00±1.00^{b}	3.32±0.28^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

The data in Table 4.33 and 4.34 indicate the effect of varying doses of *A. deserti* methanol extract given orally and intraperitoneally in Swiss albino mice after 4 weeks on red blood cells, hemoglobin and the related indices and glucose levels. As depicted from the tables, oral and intraperitoneal dosing of plant extracts to mice significantly increased levels of RBC, Hb, PCV and RDW throughout treatments after 4 weeks and were significant ($\rho \leq 0.05$) compared to control groups. Similarly, continued daily administration of herbal drug gave significant ($\rho \leq 0.05$) elevation in RBC levels including RDW and PCV indices as indicated throughout the four treatments in both routes. However, there were no significant increase in MCH and decrease in MCHC as the dose increased compared to control groups. As indicated from the tables, a significant increase in MCV values was noted compared to control groups in both routes. Oral and intraperitoneal administration showed significant ($\rho \leq 0.05$) increase in RDW that started at 450 mg/kg body weight relative to controls. Orally and intraperitoneally administratered herbal drug indicated a dose dependent ($\rho \leq 0.05$) reduction in glucose levels in comparison with control groups.

Table 4.33: Effect of *A. deserti* methanol extract given orally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment	Erythrocytes and accessible parameters							
(mg/kg bw)	RBC ($x10^{12}$/L)	Hb (g/dL)	PCV (pg)	MCH (pg)	MCHC (g/dL)	MCV (fL)	RDW (%)	GLU(mmol/L)
Control	5.60±0.54^{a}	7.98±1.73^{a}	22.36±0.89^{a}	14.27±2.72^{a}	35.96±9.42^{a}	40.33±5.29^{a}	18.70±1.47^{a}	5.64±1.20^{c}
450	6.06±0.26^{ab}	9.80±0.25^{a}	30.20±3.11^{b}	16.19±0.59^{a}	32.69±3.07^{a}	49.82±4.50^{a}	19.60±0.89^{b}	4.78±0.36^{bc}
670	7.60±1.14^{bc}	13.00±1.58^{b}	43.20±1.92^{c}	17.50±4.21^{a}	30.19±4.27^{a}	58.17±11.15^{ab}	20.80±1.10^{c}	4.05±1.44^{ab}
1000	9.18±1.29^{c}	17.60±1.67^{b}	60.00±3.08^{d}	19.63±4.16^{a}	29.38±3.04^{a}	66.59±11.56^{b}	22.40±1.52^{d}	3.83±0.96^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

Table 4.34: Effect of *A. deserti* methanol extract given intraperitoneally on erythrocytes, their accessible parameters and glucose levels of Swiss albino mice after 4 weeks

Treatment	Erythrocytes and accessible parameters							
(mg/kgbw)	RBC ($x10^{12}$/L)	Hb (g/dL)	PCV (pg)	MCH (pg)	MCHC (g/dL)	MCV (fL)	RDW (%)	GLU(mmol/L)
Control	6.06±0.37^{a}	8.98±1.33^{a}	26.53±0.95^{a}	14.91±2.68^{a}	33.85±5.65^{a}	43.93±3.24^{a}	20.20±1.10^{a}	5.38±0.36^{c}
450	7.16±0.55^{ab}	10.52±0.96^{a}	33.80±2.59^{b}	14.74±1.57^{a}	31.31±4.15^{a}	47.38±4.64^{a}	24.80±0.84^{b}	4.94±0.27^{bc}
670	8.98±0.91^{bc}	13.40±1.14^{b}	46.20±1.79^{c}	15.07±2.17^{a}	28.98±1.69^{a}	51.88±5.74^{ab}	26.80±0.84^{c}	4.44±0.65^{ab}
1000	9.18±1.91^{c}	14.80±1.30^{b}	54.00±2.74^{d}	16.52±2.70^{a}	27.45±2.61^{a}	60.65±11.52^{b}	29.80±1.48^{d}	3.68±0.33^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. Hb = hemoglobin; MCH = mean corpuscular hemoglobin; RDW= red cell distribution width-coefficient of variation; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PCV = packed red cell volume; RBC = red blood cell count; GLU = glucose levels.

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4.8.2 Analysis of white blood cells and associated parameters

The results in Table 4.35 and 4.36 indicate the effect of varying doses of *Z. usamberense* methanol extract given orally and intraperitoneally in Swiss albino mice on total leucocytes and differential leucocyte count after 4 weeks. As depicted from tables, oral and intraperitoneal administration of *Z. usamberense* extract gave statistically significant dose dependent ($\rho \leq 0.05$) elevation in WBC and MPV values across the four treatments in comparison to control groups. Oral dosing of extracts caused significant dose dependent ($\rho \leq 0.05$) increase in LYM, MON and NEU values compared to control groups. However, intraperitoneal administration indicated significant changes in LYM, MON and NEU values. As depicted from the tables, EOS values indicated significant increase compared to control groups in both routes. Oral administration showed significant increase in BAS values and significant dose dependent ($\rho \leq 0.05$) increase in intraperitoneal dosing as the extract dose increased compared to control groups. Moreover, the PLT and PDW also indicated significant increase ($\rho \leq 0.05$) in all doses of the plant extracts in all routes. The 1000 mg/kg body weight of herbal drug recorded maximal amount of WBC, EOS, MON, LYM, NEU, PLT, BAS, MPV and PDW in comparison to control groups in both routes.

Table 4.35: Effect of *Z. usamberense* methanol extract given orally on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	8.58±0.80[a]	2.78±0.53[a]	4.88±0.29[a]	0.36±0.11[a]	0.04±0.01[a]	0.52±0.08[a]	567.80±28.69[a]	15.04±0.20[a]	5.22±0.41[a]
450	9.56±0.70[a]	3.06±0.27[a]	5.38±0.58[ab]	0.44±0.09[ab]	0.06±0.01[a]	0.62±0.13[ab]	683.60±23.26[b]	16.28±0.71[b]	5.84±0.48[ab]
670	11.10±0.33[b]	3.82±0.22[b]	5.94±0.23[bc]	0.58±0.08[bc]	0.08±0.05[ab]	0.69±0.10[ab]	745.80±45.52[b]	16.48±0.59[b]	6.24±0.32[bc]
1000	12.40±0.38[c]	4.66±0.33[c]	6.14±0.28[c]	0.73±0.08[c]	0.12±0.02[b]	0.75±0.08[b]	947.80±56.14[c]	17.04±0.52[b]	6.68±0.70[c]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

Table 4.36: Effect of *Z. usamberense* methanol extract given intraperitoneally on on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	9.25±0.47[a]	3.08±0.26[a]	5.18±0.22[a]	0.54±0.09[a]	0.03±0.01[a]	0.42±0.08[a]	442.20±13.46[a]	15.46±0.40[a]	6.06±0.25[a]
450	10.63±0.22[a]	3.81±0.38[a]	5.65±0.29[ab]	0.61±0.03[a]	0.09±0.01[ab]	0.47±0.06[a]	566.00±24.34[b]	16.20±0.60[ab]	7.04±0.78[ab]
670	12.50±0.15[b]	4.94±0.21[b]	6.08±0.23[ab]	0.79±0.09[b]	0.12±0.01[b]	0.58±0.14[a]	623.60±31.88[c]	16.98±0.34[bc]	7.42±0.53[bc]
1000	15.25±1.77[c]	5.19±0.76[b]	7.49±2.10[b]	0.88±0.13[b]	0.62±0.08[c]	1.07±0.21[b]	798.20±29.34[d]	17.22±0.59[c]	8.44±0.65[c]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

The results in Table 4.37 and 4.38 indicate the effect of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally in Swiss albino mice on leucocytes and differential leucocyte count, platelets and related indices after 4 weeks. As depicted from tables, administration of *Z. chalybeum* extract gave dose dependent ($\rho \leq 0.05$) elevation of WBC and MON values in both routes. Oral and intraperitoneal administration of extract indicated significant increase in LYM, EOS, BAS and PLT values as compared to control groups. Moreover, oral administration of extract indicated significant increase in NEU values and intraperitoneal dosing revealed significant dose dependent ($\rho \leq 0.05$) increase in NEU values compared to control group. Surprisingly, herbal extracts administration indicated no statistically significant elevation in both MPV and PDW values as the dose increased compared to control groups in both routes. However, the 1000 mg/kg body weight of herbal drug gave the highest concentration of WBC, NEU, LYM, MON, EOS, BAS, PLT, MPV, and PDW in comparison to control group in both routes.

Table 4.37: Effect of *Z. chalybeum* methanol extract given orally on on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	$8.58±0.80^a$	$2.78±0.53^a$	$4.88±0.29^a$	$0.36±0.11^a$	$0.04±0.01^a$	$0.52±0.08^a$	$567.80±28.69^a$	$15.04±0.20^a$	$5.22±0.41^a$
450	$10.17±0.37^b$	$3.06±0.46^a$	$5.87±0.26^b$	$0.54±0.14^{ab}$	$0.06±0.01^a$	$0.64±0.11^a$	$1010.60±80.11^b$	$13.84±1.03^a$	$4.94±0.50^a$
670	$11.34±0.26^c$	$3.92±0.25^b$	$5.93±0.15^b$	$0.72±0.08^b$	$0.08±0.01^a$	$0.70±0.08^{ab}$	$1020.60±145.20^b$	$14.90±1.14^a$	$5.22±0.41^a$
1000	$13.02±0.46^d$	$4.04±0.38^b$	$6.11±0.45^b$	$0.94±0.09^c$	$1.02±0.01^b$	$0.91±0.17^b$	$1107.00±83.06^b$	$15.34±0.73^a$	$5.38±0.70^a$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

Table 4.38: Effect of *Z. chalybeum* methanol extract given intraperitoneally on on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	$9.25±0.47^a$	$3.08±0.26^a$	$5.18±0.22^a$	$0.54±0.09^a$	$0.03±0.01^a$	$0.42±0.08^a$	$442.20±13.46^a$	$15.46±0.40^a$	$6.06±0.25^a$
450	$10.22±0.70^a$	$3.02±0.44^a$	$5.92±0.33^{ab}$	$0.64±0.16^{ab}$	$0.08±0.05^a$	$0.56±0.29^{ab}$	$1118.00±97.84^b$	$14.72±1.82^a$	$5.08±0.46^a$
670	$12.59±0.49^b$	$4.18±0.24^b$	$6.12±0.54^b$	$0.78±0.08^{bc}$	$0.86±0.11^b$	$0.64±0.11^b$	$1210.40±166.15^b$	$15.94±0.61^a$	$6.30±1.75^a$
1000	$14.67±0.94^c$	$5.79±0.22^c$	$6.30±0.54^b$	$0.84±0.05^c$	$1.00±0.19^b$	$0.75±0.09^b$	$1262.20±52.65^b$	$16.12±0.55^a$	$6.54±0.44^a$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

The results in Table 4.39 and 4.40 indicate the effect of varying doses of *Aloe deserti* methanol extract given orally and intraperitoneally in Swiss albino mice on leucocytes and differential leucocyte count, platelets and related indices after 4 weeks. As depicted from tables, oral and intraperitoneal administration of *Aloe deserti* extract gave significant dose dependent ($\rho \leq 0.05$) elevation of WBC, LYM and EOS values in all treatments in both routes. Moreover, MPV, PLT and PDW also showed significant ($\rho \leq 0.05$) increase at all doses of the plant extracts in both routes. Oral administration led to significant dose dependent ($\rho \leq 0.05$) increase in NEU and MON values compared to control groups. Intraperitoneal administration indicated significant increasein NEU and MON as the dose increased compared to control groups. Oral treatment indicated a significant increase in BAS values and a significant dose dependent ($\rho \leq 0.05$) increase in BAS on intraperitoneal administration compared to control groups. However, the 1000 mg/kg body weight of herbal drug recorded maximum amount of WBC, NEU, LYM, MON, EOS, BAS, PLT, MPV, and PDW in comparison to control groups in the two routes.

Table 4.39: Effect of *A. deserti* methanol extract given orally on on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	8.58±0.80[a]	2.78±0.53[a]	4.88±0.29[a]	0.36±0.11[a]	0.04±0.01[a]	0.52±0.08[a]	567.80±28.69[a]	15.04±0.20[a]	5.22±0.41[a]
450	10.46±0.19[b]	3.14±0.28[a]	5.86±0.09[b]	0.48±0.08[ab]	0.06±0.01[a]	0.92±0.11[b]	696.60±4.34[b]	17.22±0.74[b]	6.92±0.36[b]
670	11.94±0.76[c]	3.78±0.16[b]	6.34±0.38[c]	0.56±0.09[bc]	0.08±0.01[ab]	1.18±1.00[b]	800.00±3.36[c]	19.60±1.52[c]	8.04±0.24[bc]
1000	14.16±0.35[d]	4.14±0.21[c]	7.72±0.26[d]	0.68±0.13[c]	0.10±0.02[b]	1.52±0.08[c]	985.00±10.74[c]	21.60±1.52[d]	10.20±1.52[c]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

Table 4.40: Effect of *A. deserti* methanol extract given intraperitoneally on on white blood cells and leucocyte count, platelets and their related parameters in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood platelets, WBC differential and associated parameters								
	WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	BAS ($x10^9$/L)	EOS ($x10^9$/L)	PLT ($x10^9$/L)	PDW (%)	MPV (fL)
Control	9.25±0.47[a]	3.08±0.26[a]	5.18±0.22[a]	0.54±0.09[a]	0.03±0.01[a]	0.42±0.08[a]	442.20±13.46[a]	15.46±0.40[a]	6.06±0.25[a]
450	11.04±0.34[a]	3.34±0.15[a]	6.28±0.25[b]	0.74±0.11[a]	0.06±0.01[b]	0.62±0.08[b]	462.60±3.78[b]	16.56±0.40[ab]	7.26±0.50[ab]
670	13.46±0.54[b]	3.64±0.11[b]	8.16±0.34[bc]	0.84±0.05[b]	0.08±0.01[b]	0.74±0.21[bc]	481.80±2.59[b]	18.08±0.29[b]	8.08±0.28[bc]
1000	15.40±1.05[c]	3.82±0.48[b]	9.80±1.10[c]	0.88±0.08[b]	0.10±0.01[c]	0.80±0.16[c]	526.60±6.43[c]	20.60±0.85[c]	9.78±0.52[c]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. BAS = basophils; NEU = Neutrophils; WBC = white blood cell count; PLT = platelets; LYM = lymphocytes; MPV = mean platelet volume; MON = monocytes; EOS = eosinophils; PDW = Platelets distribution width.

4.9 Biochemical markers

4.9.1 Analysis of biomarkers

The data in Table 4.41 and 4.42 indicate the effect of varying doses of *Z. usamberense* methanol extract given orally and intraperitoneally in Swiss albino mice on biomarkers for liver functions after 4 weeks. As depicted from tables, oral and intraperitoneal dosage of extract indicated significant dose dependent ($\rho \leq 0.05$) elevation of serum ALT, AST, ALP, GGT, LDH and CK values across the four treatments compared to control groups. In the same way, the α-AMYL values also showed significant ($\rho \leq 0.05$) elevation in all extract doses in comparison to control groups of both routes. However, 1000 mg/kg body weight of herbal drug gave the highest values for ALT, LDH, α-AMYL and CK compared to control groups in all routes. Administration of herbal drug doses resulted in significant ($\rho \leq 0.05$) reduction in I-BIL, D-BIL and T-BIL levels in all the drug doses in both routes. This decrease was significantly different from control groups. Moreover, the AST/ALT ratios statistically ($\rho \leq 0.05$) reduced as the dose increased across all treatments compared to control groups in both routes.

Table 4.41: Effect of *Z. usamberense* methanol extract given orally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	**Treatment (mg/kg bw)**			
	Control	**450**	**670**	**1000**
ALT (UL)	35.40±1.14[a]	40.40±1.95[b]	47.40±1.52[c]	54.80±1.92[d]
AST (UL)	222.80±13.75[a]	237.80±9.96[a]	254.40±4.28[b]	272.80±5.07[c]
AST/ALT	6.30±0.39[c]	5.90±0.33[bc]	5.37±0.21[ab]	4.99±0.26[a]
ALP (UL)	3.18±0.20[a]	3.72±0.26[ab]	4.00±0.31[bc]	4.70±0.84[c]
GGT (UL)	0.72±0.13[a]	0.94±0.17[a]	1.24±0.11[b]	1.53±0.13[c]
LDH (UL)	770.60±15.47[a]	843.00±5.25[b]	894.26±3.83[c]	978.40±8.44[c]
AMYL (UL)	749.60±14.79[a]	859.40±9.76[b]	906.00±9.80[c]	985.60±8.26[d]
CK (UL)	219.80±7.16[a]	244.00±04.80[b]	320.00±4.00[bc]	401.40±2.51[c]
T-BIL (µM)	11.42±0.25[b]	10.02±0.41[b]	7.34±1.37[a]	5.98±0.48[a]
I-BIL (µM)	5.12±0.44[b]	4.78±0.41[b]	3.30±0.63[a]	2.84±0.30[a]
D-BIL (µM)	6.30±0.23[d]	5.24±0.43[c]	4.04±0.76[b]	3.14±0.36[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

Table 4.42: Effect of *Z. usamberense* methanol extract given intraperitoneally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	Treatment (mg/kg bw)			
	Control	**450**	**670**	**1000**
ALT (UL)	32.00±1.58[a]	39.80±1.64[b]	43.80±1.30[c]	47.40±1.82[d]
AST (UL)	217.20±0.84[a]	229.80±9.44[b]	238.60±2.30[b]	250.20±2.95[c]
AST/ALT	6.80±0.34[c]	5.77±0.09[b]	5.45±0.18[ab]	5.29±0.24[a]
ALP (UL)	1.76±0.25[a]	2.00±0.16[a]	2.44±0.11[b]	3.20±0.16[c]
GGT (UL)	0.25±0.36[a]	0.43±0.06[ab]	0.68±0.08[b]	0.89±0.01[c]
LDH (UL)	592.60±5.49[a]	644.80±3.56[b]	792.40±5.64[c]	844.20±3.03[d]
AMYL (UL)	571.00±5.83[a]	618.20±4.92[b]	805.00±4.85[c]	998.20±6.06[d]
CK (UL)	239.60±1.49[a]	348.40±4.04[b]	256.00±1.58[c]	265.40±3.71[d]
T-BIL (μM)	10.12±0.58[c]	9.34±0.79[bc]	8.40±0.55[b]	6.92±0.43[a]
I-BIL (μM)	4.72±0.44[c]	4.40±0.47[bc]	3.94±0.29[b]	3.14±0.39[a]
D-BIL (μM)	5.40±0.69[c]	4.94±0.50[bc]	4.46±0.50[ab]	3.78±0.32[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

The data in Table 4.43 and 4.44 indicate the effect of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally in Swiss albino mice after 4 weeks on biomarkers for liver functions. As shown from tables, extracts administered in the two routes showed significant dose dependent ($\rho \leq 0.05$) increase in serum ALT values across the four treatments compared to control groups. Oral and intraperitoneal dosing indicated significant increase in AST and ALP values relative to control groups. Oral administration of extracts indicated significant dose dependent ($\rho \leq 0.05$) elevation in GGT and LDH values compared to control groups. Intraperitoneal administration indicated significant increase in GGT and LDH values relative to control groups. In the same way, the CK and α-AMYL values also showed significant increase in orally administered extracts compared to control groups. The administration of extracts indicated significant ($\rho \leq 0.05$) reduction in I-BIL, D-BIL and T-BIL levels in both

routes for the treated animals. This decrease was significantly different from control groups. However, the 1000 mg/kg body weight of herbal drug gave increased values for ALT compared to control groups in all routes. Moreover, the AST/ALT ratios significantly ($\rho \leq 0.05$) reduced in a dose dependent manner across the four treatments in comparison with control groups in both routes.

Table 4.43: Effect of *Z. chalybeum* methanol extract given orally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	**Treatment (mg/kg bw)**			
	Control	**450**	**670**	**1000**
ALT (UL)	35.40 ± 1.14^{a}	40.00 ± 1.22^{b}	45.00 ± 1.58^{b}	55.60 ± 3.65^{c}
AST (UL)	222.80 ± 13.75^{a}	258.00 ± 7.11^{b}	266.60 ± 12.01^{b}	271.80 ± 10.43^{b}
AST/ALT	6.30 ± 0.39^{b}	6.45 ± 0.20^{b}	5.93 ± 0.21^{b}	4.90 ± 0.32^{a}
ALP (UL)	3.18 ± 0.20^{a}	9.40 ± 0.56^{b}	9.58 ± 0.51^{b}	10.80 ± 1.48^{b}
GGT (UL)	0.72 ± 0.13^{a}	0.87 ± 0.08^{ab}	0.90 ± 0.05^{bc}	1.03 ± 0.06^{c}
LDH (UL)	770.60 ± 15.74^{a}	861.80 ± 16.32^{b}	929.00 ± 15.38^{c}	935.80 ± 33.52^{c}
AMYL (UL)	749.60 ± 7.16^{a}	852.80 ± 44.04^{b}	874.00 ± 13.56^{b}	884.80 ± 12.42^{b}
CK (UL)	219.80 ± 14.79^{a}	382.00 ± 41.86^{b}	395.60 ± 12.56^{b}	396.80 ± 3.27^{b}
T-BIL (µM)	11.42 ± 0.25^{d}	10.04 ± 0.56^{c}	7.96 ± 0.37^{b}	6.18 ± 0.69^{a}
I-BIL (µM)	5.12 ± 0.44^{c}	4.82 ± 0.16^{c}	3.73 ± 0.33^{b}	2.44 ± 0.34^{a}
D-BIL (µM)	6.30 ± 0.23^{c}	5.22 ± 0.54^{b}	4.24 ± 0.34^{a}	3.74 ± 0.59^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

Table 4.44: Effect of *Z. chalybeum* methanol extract given intraperitoneally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	**Treatment (mg/kg bw)**			
	Control	**450**	**670**	**1000**
ALT (UL)	32.00±1.58[a]	48.60±2.30[b]	51.00±3.39[b]	62.40±6.35[c]
AST (UL)	217.20±0.84[a]	229.80±2.09[a]	234.20±8.41[b]	246.60±7.70[b]
AST/ALT	.80±0.34[c]	4.74±0.32[b]	4.61±0.37[b]	3.98±0.30[a]
ALP (UL)	1.76±0.25[a]	10.60±2.24[b]	11.00±1.58[b]	13.20±1.30[b]
GGT (UL)	0.25±0.36[a]	0.61±0.07[b]	0.71±0.04[b]	0.94±0.06[b]
LDH (UL)	592.60±5.41[a]	593.27±9.91[a]	594.00±9.91[a]	645.80±9.39[b]
AMYL (UL)	571.00±5.83[a]	594.80±7.16[ab]	612.40±5.18[b]	694.80±37.02[c]
CK (UL)	239.60±1.14[a]	258.20±8.32[b]	288.40±7.44[b]	298.60±14.15[c]
T-BIL (µM)	10.12±0.58[d]	8.72±0.19[c]	6.16±0.34[b]	4.70±0.30[a]
I-BIL (µM)	4.72±0.44[d]	3.80±0.16[c]	3.58±0.16[b]	1.92±0.24[a]
D-BIL (µM)	5.40±0.69[c]	4.92±0.15[c]	3.58±0.23[b]	2.80±0.16[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

The results in Table 4.45 and 4.46 indicate the effect of varying doses of *A. deserti* methanol extract given orally and intraperitoneally in Swiss albino mice after 4 weeks on biomarkers for liver functions. As shown from tables, orally and intraperitoneally administratered extract showed dose dependent ($\rho \leq 0.05$) increase in serum CK, LDH. ALT and AST values across the four treatments compared to control groups. Oral administration of plant extracts showed significant dose dependent ($\rho \leq 0.05$) increase in ALP activity although there was variable increase in intraperitoneally administered dose compared to control groups. Oral dosing of extracts indicated significant increase in GGT values compared to control groups. Intraperitoneal administration revealed significant dose dependent ($\rho \leq 0.05$) increase in GGT values as dose increased relative to control groups. In the same way, the α-AMYL values also showed significant ($\rho \leq 0.05$) elevation in all herbal doses compared to control groups.

However, the 1000 mg/kg body weight herbal extracts indicated the maximum values for ALT, LDH, α-AMYL and CK compared to control groups in both routes. The administration of extracts resulted in significant ($\rho \leq 0.05$) reduction in I-BIL, D-BIL and T-BIL values in both routes. This decrease was significantly different from control groups. Moreover, the AST/ALT ratios significantly ($\rho \leq 0.05$) reduced in a dose dependent manner across all the treatments in comparison to control groups in both routes.

Table 4.45: Effect of *Aloe deserti* methanol extract given orally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	**Treatment (mg/kg bw)**			
	Control	**450**	**670**	**1000**
ALT (UL)	35.40±1.14[a]	40.80±1.30[b]	45.50±2.78[c]	48.40±1.14[c]
AST (UL)	222.80±13.75[a]	236.80±5.40[ab]	246.20±8.87[c]	254.40±6.19[c]
AST/ALT	6.30±0.39[b]	5.81±0.31[b]	5.41±0.39[a]	5.26±0.20[a]
ALP (UL)	3.18±0.20[a]	3.96±0.18[ab]	4.40±0.55[bc]	5.40±1.14[c]
GGT (UL)	0.72±0.13[a]	0.92±0.16[b]	1.06±0.21[b]	1.16±0.17[b]
LDH (UL)	770.60±15.47[a]	996.20±4.09[b]	1004.20±3.70[b]	1025.00±5.59[c]
AMYL (UL)	749.60±7.16[a]	882.00±2.70[b]	908.60±7.86[c]	1025.40±3.58[d]
CK (UL)	219.80±14.79	233.60±7.09[a]	254’00±2.55[b]	306.80±4.32[c]
T-BIL (µM)	11.42±0.25[d]	9.74±0.74[c]	8.04±0.56[b]	5.88±0.28[a]
I-BIL (µM)	5.12±0.44[d]	4.00±0.63[c]	3.16±0.30[b]	2.32±0.24[a]
D-BIL (µM)	6.30±0.23[c]	5.74±0.45[c]	4.88±0.38[b]	3.56±0.23[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey’s method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

Table 4.46: Effect of *Aloe deserti* methanol extract given intraperitoneally on biomarkers for liver function in Swiss albino mice after 4 weeks

Analytes	Treatment (mg/kg bw)			
	Control	**450**	**670**	**1000**
ALT (UL)	32.00±1.58^{a}	37.60±0.55^{b}	42.20±2.77^{c}	49.40±1.14^{d}
AST (UL)	217.20±0.84^{a}	228.00±5.70^{b}	335.20±3.11^{b}	267.00±6.67^{c}
AST/ALT	6.80±0.34^{c}	6.06±0.09^{b}	5.59±0.37^{ab}	5.41±0.12^{a}
ALP (UL)	1.76±0.25^{a}	1.96±0.24^{a}	2.02±0.19^{a}	2.44±0.21^{a}
GGT (UL)	0.25±0.36^{a}	0.52±0.08^{ab}	0.74±0.11^{bc}	1.04±0.13^{c}
LDH (UL)	592.60±5.41^{a}	635.60±8.88^{b}	765.00±5.00^{c}	846.40±4.04^{d}
AMYL (UL)	571.00±5.83^{a}	585.40±3.78^{b}	685.20±4.21^{c}	749.40±4.98^{d}
CK (UL)	239.60±1.14^{a}	288.60±1.14^{b}	300.60±2.41^{c}	344.40±4.98^{d}
T-BIL (µM)	10.12±0.58^{d}	8.62±0.29^{c}	6.38±0.19^{b}	4.92±0.11^{a}
I-BIL (µM)	4.72±0.44^{d}	3.78±0.36^{c}	2.76±0.11^{b}	1.94±0.21^{a}
D-BIL (µM)	5.40±0.69^{c}	4.84±0.23^{b}	4.62±0.26^{b}	2.98±0.26^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; GGT = γ-glutamyltransferase; CK = creatinine kinase; AST/ALT = values of aspartate transaminase and alanine transaminase activity; α-AMYL = α-amylase; T-BIL = total bilirubin; D-BIL = direct bilirubin; I-BIL = indirect bilirubin.

4.9.2 Analysis of blood metabolites

The data in Table 4.47 and 4.48 indicate the effect of varying of *Z. usamberense* methanol extract given orally and intraperitoneally after 4 weeks on kidney function markers in Swiss albino mice. Orally and intraperitoneally administered herbal extract resulted in significant dose dependent ($\rho \leq 0.05$) increase in amount of CREAT, UA and UREA levels in all the treatments in both routes compared to control groups.

Table 4.47: Effect of *Z. usamberense* methanol extract given orally on kidney function markers of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (μmol/L)	UA (μmol/L))	UREA (mM)
Control	12.52±0.85[a]	66.46±2.58[a]	3.04±0.14[a]
450	13.64±0.72[ab]	69.00±10.07[a]	4.04±0.34[b]
670	14.88±0.75[b]	90.80±13.46[b]	4.72±0.46[c]
1000	17.14±1.22[c]	118.20±7.98[c]	6.02±0.37[d]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

Table 4.48: Effect of *Z. usamberense* methanol extract given intraperitoneally on kidney function markers of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (μmol/L)	UA (μmol/L)	UREA (mM)
Control	10.44±0.83[a]	55.20±4.15[a]	3.52±0.19[a]
450	13.44±1.20[b]	60.20±1.64[a]	4.02±0.53[ab]
670	14.42±0.79[b]	65.60±1.95[b]	5.40±1.14[c]
1000	16.14±1.05[c]	73.00±2.92[c]	6.10±0.89[d]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

The results in Table 4.49 and 4.50 indicate the effect of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally after 4 weeks on kidney function markers in mice. Orally and intraperitoneally dosed herbal drug resulted in significant drug dependent ($\rho \leq 0.05$) increase in the amount of CREAT and UA values in comparison to control groups. Oral treatment showed significant dose dependent ($\rho \leq 0.05$) increase in UREA level as dose increased compared to control groups.

Table 4.49: Effect of *Z. chalybeum* methanol extract given orally on kidney function markers in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (µmol/L)	**UA (µM)**	**UREA (mM)**
Control	12.52±0.85[a]	66.46±2.58[a]	3.04±0.14[a]
450	14.40±1.14[b]	96.80±4.71[b]	3.64±0.57[ab]
670	16.40±1.14[c]	125.20±6.91[c]	4.12±0.24[b]
1000	17.60±0.89[c]	150.80±6.53[c]	5.44±0.86[c]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

Table 4.50: Effect of *Z. chalybeum* methanol extract given intraperitoneally on kidney function markers in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (µmol/L)	**UA (µmol/L)**	**UREA (mM)**
Control	10.44±0.83[a]	55.20±4.15[a]	3.52±0.19[a]
450	12.08±1.02[a]	72.40±9.53[a]	3.14±0.39[a]
670	14.22±0.77[b]	102.13±8.04[b]	3.40±0.44[a]
1000	16.60±1.14[c]	129.40±4.56[c]	4.26±0.46[b]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

The results in Table 4.51 and 4.52 indicate the effect of varying doses of *Aloe deserti* methanol extract given orally and intraperitoneally after 4 weeks kidney function markers in Swiss albino mice. Orally and intraperitoneally dosed herbal drug resulted to significant ($\rho \leq 0.05$) elevation in the amount of CREAT, UREA and UA values in both routes of administration compared to control groups.

Table 4.51: Effect of *A. deserti* methanol extract given orally on markers kidney functions in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (µmol/L)	UA (µmol/L)	UREA (mM)
Control	12.52±0.85^{a}	66.46±2.59^{a}	3.04±0.14^{a}
450	13.90±1.64^{ab}	80.20±1.92^{b}	3.44±0.62^{ab}
670	14.76±0.27^{b}	90.20±1.30^{c}	4.08±0.24^{b}
1000	15.64±0.21^{c}	100.80±3.11^{d}	4.92±3.86^{c}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

Table 4.52: Effect of *A. deserti* methanol extract given intraperitoneally on kidney function markers in Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Blood metabolites		
	CREAT (µmol/L)	UA (µmol/L)	UREA (mM)
Control	10.44±0.83^{a}	55.20±4.15^{a}	3.52±0.19^{a}
450	12.02±1.10^{ab}	60.60±1.52^{b}	4.06±0.15^{ab}
670	13.80±1.48^{bc}	68.60±0.89^{c}	4.36±0.69^{b}
1000	14.46±0.49^{c}	82.80±3.11^{c}	5.18±0.19^{c}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. UA = uric acid; UREA = Urea; CREAT = creatinine.

4.9.3 Analysis of lipid profiles

The results in Table 4.53 and 4.54 indicate the effect of varying doses of *Z. usamberense* methanol extract given orally and intraperitoneally on serum lipid profiles after 4 weeks in Swiss albino mice. The three extracts orally administerd to mice showed significant decrease in TC, HDL-C and TG in a dose dependent manner compared to the control groups ($\rho \leq 0.05$). There were no significant alterations in LDL-C levels in orally treated mice compared to normal control group. Intraperitoneally administratered extract doses caused a significant dose dependent ($\rho \leq 0.05$) reduction in LDL-C, HDL-C,TC and TG in comparison to control group.

Table 4.53: Effect of *Z. usamberense* methanol extract given orally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kg bw)	Lipid profiles			
	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Control	1.64±0.11[c]	1.44±0.11[b]	0.19±0.13[a]	1.24±0.11[c]
450	1.48±0.12[bc]	1.30±0.10[ab]	0.18±0.06[a]	0.82±0.15[b]
670	1.40±0.06[ab]	1.24±0.05[a]	0.16±0.01[a]	0.62±0.62[b]
1000	1.26±0.17[a]	1.14±0.13[a]	0.14±0.05[a]	0.48±0.08[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

Table 4.54: Effect of *Z. usamberense* methanol extract given intraperitoneally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Lipid profiles			
	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Control	1.67±0.40[c]	1.50±0.39[b]	0.17±0.02[c]	0.78±0.13[b]
450	1.60±0.15[b]	1.00±0.16[a]	0.15±0.03[bc]	0.60±0.07[a]
670	0.95±0.09[b]	0.82±0.08[a]	0.13±0.01[b]	0.50±0.07[a]
1000	0.64±0.11[a]	0.64±0.11[a]	0.10±0.01[a]	0.44±0.09[a]

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

The results in Tables 4.55 and 4.56 indicate the effect of varying doses of *Z. chalybeum* methanol extract given orally and intraperitoneally on serum lipid profiles after 4 weeks in Swiss albino mice. Administration of the extract doses to mice demonstrated significant ($\rho \leq 0.05$) reduction of TC, HDL-C, LDL-C and TG levels in both routes compared to control groups.

Table 4.55: Effect of extract *Z. chalybeum* methanol extract given orally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Lipid profiles			
	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Control	$1.64±0.11^{b}$	$1.44±0.11^{b}$	$0.24±0.13^{b}$	$1.24±0.11^{c}$
450	$1.72±0.08^{b}$	$1.50±0.16^{b}$	$0.23±0.11^{b}$	$1.06±0.13^{bc}$
670	$1.12±0.36^{a}$	$1.00±0.40^{a}$	$0.20±0.44^{a}$	$0.88±0.08^{b}$
1000	$0.93±0.10^{a}$	$0.84±0.09^{a}$	$0.19±0.02^{a}$	$0.51±0.14^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

Table 4.56: Effect of *Z. chalybeum* methanol extract given intraperitoneally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Lipid profiles			
	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Control	$1.67±0.40^{b}$	$1.50±0.39^{b}$	$0.17±0.02^{c}$	$0.78±0.13^{c}$
450	$1.10±0.19^{a}$	$1.02±0.19^{a}$	$0.08±0.01^{b}$	$0.51±0.02^{b}$
670	$0.92±0.12^{a}$	$0.86±0.11^{a}$	$0.06±0.01^{a}$	$0.42±0.08^{b}$
1000	$0.68±0.17^{a}$	$0.64±0.17^{a}$	$0.04±0.01^{a}$	$0.26±0.05^{a}$

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

The results in Table 4.57 and 4.58 indicate the effect of varying doses of *Aloe deserti* methanol extract given orally and intraperitoneally on serum lipid profiles after 4 weeks in Swiss albino mice. Oral administration resulted to dose dependent ($\rho \leq .05$) decrease in TC, HDL-C, LDL-C and TG levels compared to control groups. Intraperitoneal dosing indicated significant decrease in levels in TC, HDL-C and TG, and no significant alteration in LDL-C compared to control groups.

Table 4.57: Effect of *A. deserti* methanol extract given orally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Lipid profiles			
	TC (mmol/L)	**HDL-C (mmol/L)**	**LDL-C (mmol/L)**	**TG (mmol/L)**
Control	1.64 ± 0.11^{c}	1.50 ± 0.10^{c}	0.32 ± 0.08^{c}	1.24 ± 0.11^{c}
450	1.82 ± 0.15^{c}	1.44 ± 0.11^{bc}	0.24 ± 0.13^{bc}	0.83 ± 0.09^{b}
670	1.40 ± 0.10^{b}	1.26 ± 0.11^{b}	0.14 ± 0.05^{b}	0.72 ± 0.08^{b}
1000	0.91 ± 0.09^{a}	0.82 ± 0.08^{a}	0.10 ± 0.02^{a}	0.56 ± 0.05^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

Table 4.58: Effect of *A. deserti* methanol extract given intraperitoneally on lipid profiles of Swiss albino mice after 4 weeks

Treatment (mg/kgbw)	Lipid profiles			
	TC (mmol/L)	**HDL-C (mmol/L)**	**LDL-C (mmol/L)**	**TG (mmol/L)**
Control	1.67 ± 0.40^{b}	1.50 ± 0.39^{b}	0.18 ± 0.02^{a}	0.78 ± 0.13^{b}
450	1.86 ± 0.15^{b}	1.68 ± 0.08^{ab}	0.17 ± 0.08^{a}	0.82 ± 0.10^{b}
670	1.50 ± 0.23^{ab}	1.38 ± 0.25^{ab}	0.16 ± 0.04^{a}	0.46 ± 0.09^{a}
1000	1.15 ± 0.15^{a}	1.06 ± 0.15^{a}	0.14 ± 0.01^{a}	0.36 ± 0.04^{a}

Findings are presented as Mean ± Standard Deviation per group (5 animals). Independent one-way ANOVA was used to differentiate the groups. Means of individual groups with different superscript character determined using Tukey's method at $\rho \leq 0.05$ are significant. TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein; HDL-C = high density lipoprotein.

4.10 Phytochemicals analysis

The methanol extracts of *Zanthoxylum usambarense*, *Zanthoxylum chalybeum* and *Aloe deserti* plants revealed the presence of various bioactive compounds at varying intensities (Table 4.59). The *Zanthoxylum usambarense* plant extracts contained biologically active components such as alkaloids, flavonoids, terpenoids, tannin and glycosides. There were moderate levels of anthraquinones and reducing sugars but indicated absence of saponins. The *Zanthoxylum chalybeum* indicated presence of high tannins and moderate alkaloids, flavonoids, anthraquinones and glycosides. It also indicated low levels of terpenoids, reducing sugars but indicated absence of

saponins. The methanol extract of *Aloe deserti* plant had no saponins and flavonoids but showed moderate tannins and low levels of alkaloids, terpenoids, anthraquinones, glycosides and reducing sugars.

Table 4.59: Phytochemical analysis of the methanol plant extracts

Phytochemicals	Tests	*Zanthoxylum usamberense*	*Zanthoxylum chalybeum*	*Aloe deserti*
Alkaloids	Dragendroff's test	+++	++	+
Terpenoids	Salkowski's test	+++	+	+
Saponins	Frothing test	-	-	-
Flavanoids	Alkaline test	+++	++	-
Tannins	Ferric chloride test	+++	+++	++
Anthraquinones	Borntrager's test	++	++	+
Glycosides	Salkowski's test	+++	++	+
Reducing sugars	Fehling's test	++	+	+

Key: +++ (high), ++ (moderate), + (low), – (Absent)

4.11 Analysis of trace metals

Heavy metals and trace metals were quantitatively analyzed using Atomic Absorption Spectrophotometer (AAS). The results (Table 4.60, 4.61 and 4.62) indicated presence of macro-nutrients, micro-nutrients and the toxic heavy metals of *Zanthoxylum usamberense*, *Zanthoxylum chalybeum* and *Aloe deserti* plants used in the treatment of gastroenteritis. The results indicated that the three plant extracts contain the macro-minerals such as potassium (K), magnesium (Mg) and calcium (Ca) and micro-minerals such as manganese (Mn), zinc (Zn), copper (Cu), iron (Fe) and the toxic heavy metals such as lead (Pb), chromium (Cr), strontium (Sr) Vanadium (V), Mercury (Hg) and arsenic (As) at differing concentrations.

The examination of the data shows that plant extracts provided macro-minerals (K, Mg, Ca) and micro-minerals (Zn, Cu, Fe) and toxic metals (Pb, Cu, Sr, V, Hg, As) below the recommended daily requirements except Mn and Cr in mice models in all

the studied plants. The *Zanthoxylum usamberense* plant extracts yielded the highest amount of Mg, K and Ca followed by *Zanthoxylum chalybeum*. All extracts had no detectable amount of V and Hg at all doses. The *Zanthoxylum chalybeum* extracts had undetectable amount of Cu at all dosage used. *Aloe deserti* extracts had undetectable amount of Pb and Sr at all dosage administered. Toxic heavy metal such As, Hg, V and Pb were below the RDA of mouse.

Table 4.60: Essential phyto-elements given to mouse per day

Elements (µg/g)	450 mg/kg of methanol herbal extracts given per day (µg/day)			
	Zanthoxylum usamberense	*Zanthoxylum chalybeum*	*Aloe deserti*	RDA for mouse (µg/day)
Mg	144.00±0.20 **1.51×10^{-5}**	123.00±0.05 **1.66×10^{-5}**	111.91±0.23 **1.94×10^{-5}**	171.4
K	45.00±0.62 **6.59×10^{-6}**	33.00±2.55 **6.71×10^{-6}**	43.00±3.00 **2.96×10^{-5}**	75
Ca	37.71±0.43 **2.62×10^{-6}**	28.78±0.24 **3.89×10^{-6}**	22.02±9.34 **4.82×10^{-6}**	428.6
Mn	4.13±0.04 **4.39×10^{-7}**	2.34±0.05 **3.16×10^{-7}**	3.25±0.05 **5.58×10^{-7}**	1.07
Fe	2.02±0.08 **1.95×10^{-5}**	1.45±0.08 **1.51×10^{-5}**	1.32±0.54 **1.66×10^{-5}**	10.7
Zn	2.44±0.37 **5.27×10^{-7}**	0.94±0.34 **1.27×10^{-7}**	3.90±0.42 **3.29×10^{-7}**	4.714
Pb	0.06±0.03 **2.70×10^{-9}**	0.02±0.03 **2.70×10^{-9}**	BDL **8.10×10^{-9}**	0.82
Cr	0.94±0.16 **1.35×10^{-8}**	0.03±0.06 **4.05×10^{-9}**	0.10±0.15 **1.27×10^{-7}**	0.015
Sr	0.11±0.83 **2.48×10^{-8}**	0.24±0.27 **3.24×10^{-8}**	BDL **1.48×10^{-8}**	10.71
Cu	0.27±0.07 **5.40×10^{-8}**	BDL **1.08×10^{-8}**	0.42±1.41 **3.65×10^{-8}**	0.729
As	0.03±0.17 **0.82×10^{-8}**	0.05±0.23 **0.54×10^{-8}**	0.02±0.05 **0.81×10^{-9}**	0.08
V	BDL **5.43×10^{-8}**	BDL **5.03×10^{-8}**	BDL **2.35×10^{-8}**	2.10×10^{-5}
Hg	BDL **3.40×10^{-8}**	BDL **4.14×10^{-8}**	BDL **2.24×10^{-8}**	2.50×10^{-5}

Findings are presented as Mean ± Standard Deviation of extract replicates. BDL= mineral levels below the limit of detection by AAS. First row indicates levels of minerals in herbal drug (µg/g). Second row shows given minerals every day. RDA = required daily allowance for mouse (Chandaka, 2017)

Table 4.61: Essential phyto-elements given to mouse per day

Elements (µg/g)	670 mg/kg of methanol herbal extracts given per day (µg/day)			
	Zanthoxylum usamberense	*Zanthoxylum chalybeum*	*Aloe deserti*	RDA for mouse (µg/day)
Mg	144.00±0.20 **2.25×10^{-5}**	123.00±0.05 **2.47×10^{-5}**	111.91±0.23 **2.89×10^{-5}**	171.4
K	58±0.82 **9.81×10^{-6}**	35±2.55 **9.99×10^{-6}**	47.00±2.00 **4.41×10^{-5}**	75
Ca	37.71±0.43 **3.90×10^{-6}**	28.78±0.24 **5.79×10^{-6}**	22.02±9.34 **7.18×10^{-6}**	428.6
Mn	3.13±0.04 **6.53×10^{-7}**	2.37±0.05 **4.71×10^{-7}**	3.26±0.05 **8.31×10^{-7}**	1.07
Fe	4.02±0.08 **3.78×10^{-5}**	2.45±0.08 **2.24×10^{-5}**	2.22±0.44 **2.47×10^{-5}**	10.7
Zn	2.44±0.37 **7.85×10^{-7}**	0.94±0.34 **1.89×10^{-7}**	3.90±0.42 **4.90×10^{-7}**	4.714
Pb	0.06±0.03 **2.70×10^{-9}**	0.02±0.03 **4.02×10^{-9}**	BDL **1.21×10^{-8}**	0.82
Cr	0.94±0.16 **2.01×10^{-8}**	0.05±0.06 **6.03×10^{-9}**	0.12±0.15 **1.89×10^{-7}**	0.015
Sr	0.11±0.83 **3.62×10^{-8}**	0.24±0.27 **4.82×10^{-8}**	BDL **2.21×10^{-8}**	10.71
Cu	0.27±0.07 **8.04×10^{-8}**	BDL **1.61×10^{-8}**	0.12±0.04 **5.43×10^{-8}**	0.729
As	0.02±0.16 **1.21×10^{-8}**	0.01±0.13 **0.80×10^{-8}**	0.02±0.03 **1.21×10^{-9}**	0.08
V	BDL **5.43×10^{-8}**	BDL **5.03×10^{-8}**	BDL **2.35×10^{-8}**	2.10×10^{-5}
Hg	BDL **4.44×10^{-8}**	BDL **4.55×10^{-8}**	BDL **2.45×10^{-8}**	2.50×10^{-5}

Findings are presented as Mean ± Standard Deviation of extract replicates. BDL= mineral levels below the limit of detection by AAS. First row indicates levels of minerals in herbal drug (µg/g). Second row shows given minerals every day. RDA = required daily allowance for mouse (Chandaka, 2017)

Table 4.62: Essential phyto-elements given to mouse per day

Elements (µg/g)	1000 mg/kg of methanol herbal extracts given per day (µg/day)			
	Zanthoxylum usamberense	*Zanthoxylum chalybeum*	*Aloe deserti*	RDA for mouse (µg/day)
Mg	144.00±0.20	123.00±0.05	111.91±0.23	171.4
	3.36×10^{-5}	**3.69×10^{-5}**	**6.58×10^{-5}**	
K	61±0.69	42±1.62	50.00±2.00	75
	1.46×10^{-5}	**1.49×10^{-5}**	**2.96×10^{-5}**	
Ca	37.71±0.43	28.78±0.24	22.02±9.34	428.6
	5.82×10^{-6}	**8.64×10^{-6}**	**1.07×10^{-5}**	
Mn	4.13±0.04	2.34±0.05	3.25±0.05	1.07
	9.75×10^{-7}	**7.02×10^{-7}**	**1.24×10^{-6}**	
Fe	6.02±0.06	3.45±0.29	3.62±0.44	10.7
	4.32×10^{-5}	**3.35×10^{-5}**	**3.69×10^{-5}**	
Zn	2.44±0.37	0.94±0.34	3.90±0.42	4.714
	1.17×10^{-6}	**2.82×10^{-7}**	**7.31×10^{-7}**	
Pb	0.06±0.03	0.02±0.03	BDL	0.82
	6.00×10^{-9}	**6.00×10^{-9}**	**1.80×10^{-7}**	
Cr	0.94±0.16	0.03±0.06	0.10±0.15	0.015
	3.00×10^{-8}	**9.00×10^{-9}**	**2.82×10^{-7}**	
Sr	0.11±0.83	0.24±0.27	BDL	10.71
	5.40×10^{-8}	**7.20×10^{-8}**	**3.31×10^{-8}**	
Cu	0.27±0.07	BDL	0.12±1.41	0.729
	1.20×10^{-7}	**2.40×10^{-8}**	**8.11×10^{-8}**	
As	0.04±0.17	0.07±013	0.06±0.03	0.08
	1.80×10^{-8}	**1.20×10^{-8}**	**1.80×10^{-8}**	
V	BDL	BDL	BDL	2.10×10^{-5}
	8.10×10^{-8}	**7.50×10^{-8}**	**3.51×10^{-8}**	
Hg	BDL	BDL	BDL	2.50×10^{-5}
	4.43×10^{-8}	**3.03×10^{-8}**	**1.35×10^{-8}**	

Findings are presented as Mean ± Standard Deviation of extract replicates. BDL= mineral levels below the limit of detection by AAS. First row indicates levels of minerals in herbal drug (µg/g). Second row show given minerals every day. RDA = required daily allowance for mouse (Chandaka, 2017)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMEDATIONS

5.1 DISCUSSION

The toxic effects of synthetic antibiotics and emergence of resistant pathogens has captivated the study of active biomolecules from plant species used traditionally as herbal remedies (Kaigongi, 2014). The herbal extracts provide potential source of new antimicrobial drugs for managing resistant pathogenic bacteria (Kone, 2004). Therefore, there is a need to explore antimicrobial activity and toxicity of herbal extracts as an alternative strategy to stop the spread of drug-resistant micro-organisms. The present study was designed to evaluate the *in vitro* antimicrobial activity and *in vivo* toxicity of the *Z. usambarense, Z. chalybeum* and *A. deserti* plants used in Meru County to manage bacteria that cause gastroenteritis by local communities.

Upon examination of the data from this study, *Z. usambarense* extracts were active and effective against all the tested Gram-positive microbes and Gram-negative *Escherichia coli* ATCC 25922 except the Gram-negatives such as *Shigella dysentireae* ATCC 13313 and *Salmonella typhi* ATCC 19430. Findings from other researchers demonstrates that Gram-negative bacteria possess an outer membrane which is absent in Gram-positive bacteria (Nguyen, 2015). This membrane acts as a permeability barrier and does not allow antimicrobial agents to access their target in bacterial cells making Gram negative bacteria resistant to herbal drugs (Nguyen, 2015). The highest inhibition of *Z. usambarense* was shown in *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29912 and least activity against *E. coli* ATCC 25922

The *Z. chalybeum* methamol extract was only active against *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 10876 with weak susceptibility. This signifies that *Z. usambarense* and *Z. chalybeum* have potential antimicrobial activity for management of bacterial gastroenteritis. The inhibition growth of tested bacteria by methanol extracts of *Z. usambarense* and *Z. chalybeum* indicates that these plant extracts contain phyto-antimicrobial agents which are active against tested pathogenic micro-organisms. This is appertained to the presence of bioactive constituents like alkaloids, tannins, flavonoids, anthraquinone, terpenoids and glycosides. These phytochemicals are known to be biologically active and therefore aid the antimicrobial activity (Abiba, 2013).

Findings from this study revealed that *Aloe deserti* did not show activity against any of the tested micro-organisms. This implies that extracts of *Aloe deserti* plant had no flavonoids and glycosides but showed the existence of alkaloids, tannins and anthroquinones. The contrasting results on antibacterial activities of other *Aloe* species such sash as *Aloe secundiflora* could be due to differences in bioassay techniques used (Mariita, 2010). According to Mariita (2010), phytochemical analysis of *Aloe secundiflora* revealed presence of flavonoids suggesting a rich source of antibacterial activity against *S. typhi* and *E. coli*. These contrasting of results could also be attributed to the locality of the plant species, storage conditions and test micro-organisms used in some of those studies. The locality modifies plants influencing presence or absence of certain phytochemicals (Farhat, 2011).

The plants that contain tannins and flavonoids compounds have been reported to reveal antimicrobial activity against both Gram-negative such as *Escherchia coli* and

Gram-positives such as *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis* (Nguyen, 2015; India, 2015) and therefore acts as broad-spectrum antibacterial compounds. Alkaloids and flavonoids have been reported to be active against both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*, *B. cereus*, *S. aureus* and *E. faecalis* bacteria (Abiba, 2013).

The antimicrobial activity of flavonoids occurs through several mechanisms such as inhibition of deoxyribonucleic acid synthesis, interfering with synthesis of bacterial cell wall and interfering with protein synthesis (Nguyen, 2015). Another inhibitory effect of flavonoids is the reduction of bacterial cell membrane fluidity (reducing permeability or diffusion of molecules) (Holten, 2000). The inhibition growth of pathogens as result of tannins occurs through formation of irreversible complexes that inhibit cell protein synthesis (Bhawana, 2015). It is reported that tannins contain astringent (precipitate protein) used to treat intestinal disorders such as diarrhea supporting treatment for bacterial gastroenteritis (Bhawana, 2015). Moreover, this phyto-antimicrobial agent inhibits energy metabolisms necessary for biosynthesis in bacteria (Nguyen, 2015). This kind of explanation support results of this study, that secondary metabolites such as flavonoids and tannins are responsible for antimicrobial property.

The MIC of methanol plant extracts provides the potency of the phyto-antimicrobial agents against pathogenic micro-organisms (Korir, 2012). It was tested in this study at different concentrations to understand the most effective concentration. The lower the MIC, the better the herbal drugs against the microbes tested (Korir, 2012). The plant extracts of low concentrations, shorten the length of treatment, reduces cost of

treatment, and reduces drug overdose and toxicity and/or the side effects (Kitonde, 2013). In this study, plant extracts gave MBC that was greater or equal to MIC against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 29299, *Enterococcus faecalis* ATCC 29912 and *Escherchia coli* ATCC 25922. This clearly indicates that the concentration that killed *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29912 and *Escherchia coli* ATCC 25922 is greater than that inhibited growth of test micro-organisms.

The extracts showing high MBC/MIC ratios exhibits lowest antimicrobial activity (bacteriostatic activity) (India, 2015). The plant extracts that exhibited MBC/MIC values less than 4, their MIC showed the bactericidal action (India, 2015). The bacteriostatic agents only inhibit growth of bacteria (India, 2015). There is no killing of pathogenic bacteria leading to emergence of new drug-resistant bacteria (disease causing micro-organisms) (Abiba, 2013). The bactericidal compounds avert the emergence of resistant pathogenic microbes by killing them (Anantharaman, 2010). The killing is by destroying or rapturing cell membrane, causing cellular contents leakage and finally dying of bacteria (Marzouk, 2010).

The methanol extracts of *Z. usambarense* is effective against Gram-positive bacteria and less active against Gram-negative bacteria. The extra cell membrane outside the cell wall in Gram-negative bacteria (India, 2015) acts as a permeability barrier, limiting the access of phyto-antimicrobial agents to their targets site in the bacterium cell (Nguyen, 2015). The phyto-antibacterial agents of *Z. usambarense* are more active towards Gram-positive bacteria such as *B. subtilis* ATCC 6633, *B. cereus*

ATCC 10876, *and S. aureus* ATCC 29213 and less active against Gram-positive bacteria such as *E. faecalis* ATCC 29912 and Gram-negative such as *E. coli* ATCC 25922. The *Z. usambarense* extracts exhibited low MBC/MIC values (bactericidal) and acted as broad spectrum anti-microbial agent.

The *in vitro* time kill assay provides information on the pharmacodynamics (speed of extracts bactericidal activity) of herbal drugs and/or antibiotics (Abiba, 2013; Sim, 2014). In the present study, only extracts with high antibacterial activity towards tested bacteria (MIC≤50 mg/ml), were used for time kill kinetics studies. The time kill exposure of tested micro-organism was examined at 0.5×MIC, 1×MIC and 2×MIC of extracts concentrations (Sim, 2014). The time kill assay of the bio-active compounds on the tested micro-organisms showed methanol extract of *Z. usambarense* at 0.5×MIC against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29912 acknowledged the reality of bacteria whose prevention was bacteriostatic after four-hour contact, but the bacteria regrew to the same levels as the control inoculum after 6, 8 and 24 hours. Similarly, regrowth occurred in *Escherichia coli* ATCC 25922 after four hours' exposure to extract at 0.5×MIC to the same levels as the control colonies after 24 hours. This indicated that pathogenic bacteria are resistance to the antibacterial action of herbal drugs at low concentrations (bacteriostatic).

The methanol of *Z. usambarense* extracts at 1×MIC against *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 10876 revealed existence of bacteria whose inhibition was bactericidal within six and eight-hour contact leading 99.9% killing of colonies after 24 hr respectively. But the regrowth occurred in *Enterococcus faecalis*

29912 and *E. coli* ATCC 25922 after four hr exposure to the same levels as the control colonies after 24 hours. This indicated that bacteria are resistance to the antibacterial action at medium herbal drugs concentrations. Aggressive bactericidal activities for *Z. usambarense* extracts were achieved at concentrations higher than MIC, for example, 2×MIC against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* ATCC 29213 completely killed bacteria (99.9%) colonies within six and eight hours. In addition, methanol extracts of *Z. usambarense* plant at 2×MIC reduced (74.2%) Enterococcus *faecalis* ATCC 29912 within twenty-four hours upon which it became 174.67±5.51cfus after 24hours.

At 0.5×MIC and 1×MIC values, the *Z. usambarense* plant extract showed bacteriostatic activity against (*Enterococcus faecalis* ATCC 29912 and *Escherichia coli* ATCC 25922) microbes and at 2×MIC value, plant extract showed rapid bactericidal activity to tested Gram-positive bacteria only (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 29213). The *Z. usambarense* extract exhibited time dependent killing of Gram-positive bacteria only. The bioactive compounds that demonstrated faster killing of bacteria basically eliminate bacterial infections expeditiously than those exhibiting slow killing of bacteria (Abiba, 2013).

In slow killing drug concentration, bacteria can survive beyond 24 hr period of exposure in antibiotics (Abiba, 2013) and develops into stabilized L-forms which regrows to vegetative form after inactivating drugs or destroying of antibiotics working mechanisms (Abiba, 2013). There are two categories of L-form bacteria. The Unstable L-form bacteria are able to divide and return to original bacteria cell

morphology. Others are Stable L-form bacteria which are not able to revert to initial cell morphology. The two form of bacteria develops from both Gram-positive and Gram-negative bacteria (Leaver, 2009). In this study, *Z. usambarense* extracts of concentrations MIC and 0.5×MIC, initially prevented growth of bacteria up to six and others eight hours and thereafter promoted their regrowth to the same levels as the control colonies after 24 hours. This phenomenon is common in bacteria resistant to drugs/antibiotics. Bacteria remains viable and even regrow or multiply to vegetative form after the antibiotics is inactivated completely (Abiba, 2013). Other bacteria were killed completely by herbal drug. The phytochemical compounds present in *Z. usambarense* plant are responsible for the inhibition or killing of tested Gram-positive and few Gram-negative bacteria and this advocates for its use as an alternative therapy or remedy in management of pathogenic bacteria that cause bacterial gastroenteritis in humans.

The continued use of *Zanthoxylum usambarense, Zanthoxylum chalybeum* and *Aloe deserti* to manage pathogenic bacteria that cause bacterial gastroenteritis traditionally, little is known about their toxicity. This is why *in vivo* toxicity study or toxicological analysis of herbal extracts is evaluated to judge their safety for therapeutic uses in humans. The toxicity is an expression of being poisonous or harmful as a result of interaction between drug toxicants and animal body cells (Subramanion, 2011). The poisonous state of the drug is dependent on the route of administration and site of exposure in animal body, which is oral, intraperitoneal, intravenous or intramuscular (Abiba, 2013). The site of interaction can occur on cell surface, within cell or in body tissues. The toxicity manifests itself after toxicants bind with vital organs such as

kidney, spleen, lungs, heart, brain and liver. Once toxic, drugs become unsuitable for therapeutic purposes in humans (Subramanion, 2011).

In this study, the toxicity of *Zanthoxylum usambarense, Zanthoxylum chalybeum* and *Aloe deserti* extracts was assessed in Swiss albino mice at varying doses of 450, 670, and 1000 mg/kg body weight and compered with that of control groups to recommend for their suitability use. In the first week of oral dosing, mice showed reduced consumption of foods. This was probably due to bitter taste of herbal drugs but later normalized for the rest of three weeks. In both route, no mortality, no abnormal behavior was observed in all animals. This implies that plant extracts are safe for therapeutic purposes. Untreated groups showed no physical changes in their body appearance.

The weight of body, organ weight and relative organ weight are the good indicators for toxicity in human beings (Osafanme, 2020). All experimental animals showed increase in body weight with much differences between the treated and control groups. The reduced growth of the mice compared to that of control groups indicates that *Zanthoxylum usambarense, Zanthoxylum chalybeum* and *Aloe deserti* extracts contained anorexigenic compounds (substances) which reduced or delayed growth of mice models in the treated groups.

Herbal extracts contain a variety of secondary metabolites (Ikpeme, 2012). Some of the secondary metabolites present in *Zanthoxylum usambarense, Zanthoxylum chalybeum* extracts include alkaloids, terpenoids, tannins, flavonoids, glycosides and anthraquinones (Antal, 2009). The *Aloe deserti* extracts contained tannins, alkaloids,

terpenoids, anthraquinones and glycosides only. The phyto-chemical substance such as tannins has been reported to reduce feed intake by interfering with animal appetite and reducing chemical breakdown of feed in the gut (Ababa, 2013). Tannins have component astringent that has been used traditionally to manage intestinal disorder such as diarrhea (Banana, 2015). Once tannin is taken, it causes astringency which is the sensation of tannin-salivary glycoprotein complexes (Akinnuga, 2011). The astringency retards body growth by reducing food taken, inhibiting food digestion and incorporation of absorbed nutrients into the animals' body (Abiba, 2013). This facilitated to decreased body weight gain in the herbal drug treated animals. The weight gains on weekly basis significantly ($p \leq 0.05$) reduced when dose of extracts increased as compared to control groups for 28 days in mice experimental models.

In addition, flavonoids present in plant extracts interferes with action of several enzymes due to antioxidant properties (Teshager, 2016). This shows that herbal extracts caused alterations in various food metabolisms in extracts treated experimental animals. It also indicates that herbal extracts interfered with nutritional benefits (Osafanme, 2020) (e.g. body and organ weight gains) as expected from animals supplied with food and water ad libitum. There were decreased assimilation of absorbed nutrients such as glucose, amino acids and fatty acids to body tissues (Sanchanta, 2005). The plant extracts significantly reduced weight of vital organs such as lungs, spleen, liver, brain, heart and kidney, thus revealing possible toxic effects to these organs in the treated animals (Osafanme, 2020).

The plants secondary metabolites blocked fat-glucose absorption in the site of respiration (mitochondria) (Salawu, 2008). This blockage interfered with energy

expenditure in treated animals. Other metabolites also increase rapid oxidation of glucose as well enhancing proteolysis of skeletal muscles (Abiba, 2013). This explains the decrease in the weight of various body organs such as brain, kidney, spleen and lungs in all extracts (Osafanme, 2020). Only *Aloe deserti* extracts decreased the organ weight of liver, testes and heart in both routes (Table 4.22). There were percentage organ weight reduction especially vital organs such as spleen and lungs in intraperitoneal dosing of *Zanthoxylum usambarense* plant extracts. Also extracts of *Zanthoxylum chalybeum* pant reduced percentage organ weight of vital organs such as kidney, spleen and lungs However, the dosing of 450 mg/kg to weight of body daily had minimal changes on the weight of organ and percentage weight of lungs, heart, testes, kidney, spleen, liver and brain to animals' body weight.

The effects of plant extracts on hematological parameters were examined in this study. Hematological analysis is used to access the toxicity of foreign compounds on blood composition (Baker, 2021). The hematology blood count determines the effect of phytochemicals on hematopoietic system (Subramanion, 2011). Findings from this study showed that the extract administered at dosage used had effect on circulating erythrocytes (or the hematopoiesis). High doses gave important boost in RBCs, PCV, MCV, Hb, MCH, RDW and remarkable reduction MCH quantities in all the treated animals in both administration routes. The increase in red blood cell, mean cell volume, packed cell volume, hemoglobin concentration and red cell distribution width was induced by daily administration of 670 mg/kg up to 1000 mg/kg body weight of herbal drugs in all administered routes. This dose-dependence phenomenon also explains the effect of phytochemicals on hematopoietic system in such a way that the

higher dose of the extracts increased the production of blood cells (erythropoiesis) involved in transport of more oxygen in tissues (Baker, 2021).

The increased levels of RBCs, PVC and Hemoglobin correlate with the absolute population of erythrocytes (Salawu, 2008). The increased PVC shows a better transportation of oxygen and thus results in increased oxygen supply in body tissues (polycythemia) (Ayman, 2013). The increased red blood cells could have been induced by tissue hypoxia in body organs and this is used in diagnosing of anemic conditions (Barger, 2003). The administered plant extracts facilitated the release of the hormone erythropoietin, a glycoprotein which stimulates the production of red blood cells.

The doses of methanol extracts of *Zanthoxylum usambarense, Zanthoxylum chalybeum* and *Aloe deserti* administered in Swiss albino mice for weeks significantly increased leukocytes (neutrophils, lymphocytes, eosinophils and monocytes) and platelets in dose-dependent manner across the four treatments in both routes. The increase (leukocytosis) shows response of the different tissues to foreign antigen, injuries or sign of underlying toxicity due administration herbal extracts (Njagi, 2015) and bacterial infections in animal bodies (Sanshanta, 2005). This indicates herbal extracts contain glycosides that enhanced the ability to boost the immune defense system in experimental animals (Antal, 2009). Glycosides have anti-inflammatory property and so has vital effect on acute inflammatory processes in body (Baker, 2021). The plant extracts stimulate leucopoiesis, that is, yellow bone marrow undergoes cell division, differentiates and maturates giving rise to committed stem cells that lead to production of erythrocytes (Salasanti, 2014). In addition to this, plant extracts may possess lymphocytic activities that facilitate lymphocytes (B-cells and T-

cells) to produces antibodies. The produced T-lymphocyte cells boosting immune system of the patients (Thelma, 2020). Extracts that contain glycosides boost lymphocytic activities suitable to people attacked by opportunistic diseases (Mariita, 2010). This makes the three-plant studied suitable in immunocompromised HIV/AIDS patients.

The increased number of white blood cells could be a response of body to defensive mechanism to bacterial infections, atrophic and tissue injuries following the cytotoxic effect of the drugs (Akinnuga, 2011). Other blood components such as platelets are responsible for blood clotting mechanism (Abiba, 2013). The significant increase in platelets (cells responsible for clotting process upon tissue injury) suggests that plant extracts have a compound that promotes thrombocytosis/ hemostasis (Baker, 2021) (formation of clot that stops bleeding during clotting mechanism). This could be as result of flavonoids and alkaloids which boost immune mechanism in the body (Sanchanta, 2005). The administration of plant extracts in both routes decreased amount of MCHC in all treatments and this suggest reducing oxygen supply to tissues resulting to tissue hypoxia (Abiba, 2013). This causes vital organs such as liver, spleen and kidney to increase in size and cells swelling continue. The swollen organs rapture releasing their contents outside, resulting to reduced organ size or decreased organ weights (Abiba, 2013).

Because of tissue hypoxia, body cells start relying only on glycolysis pathway for energy production (Abiba, 2013). The body cells rapidly deplete phosphocreatine (responsible for ATP production) and glycogen (Salasanti, 2014). The ATP production decreases leading to disruption of the Na^+ - pump that control osmotic

potential, which is a tendency of solution to withdraw water across cell membrane (Baker, 2021). This results to swelling of cells and its organelles (Raza, 2002). The swollen cell forces its contents/inclusion to leak through permeable (porous) cell membrane to the blood circulatory system (Anatal, 2002). The decreased intercellular pH during anaerobic glycolysis allows the release of lysosomal enzymes (Subramanion, 2011). This lytic enzyme damages cell membrane leading to release of cell contents and results to irreversible cell damage (Abiba, 2013).

Injury in organs resulting from tissue hypoxia is responsible for reduced organ size in the all treated animals (Tiwari, 2010). The damages of organs could be as a result of altered serum levels (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, lactate dehydrogenase, creatine kinase and amylase) administered in mice orally and intraperitoneally with methanol extracts (*Z. usambarense, Z. chalybeum* and *Aloe deserti*) daily for 4 weeks. The mentioned organs get rid of drugs and are directly exposed to cytotoxic effects of exogenous compounds during metabolisms (Abiba, 2013). Animal shows probable clinical symptoms evoked by the organic compounds extracted from plants under study (Subramanion, 2011).

In this study, it was therefore reasonable to examine the toxicity (safety) of herbal extracts in vital organs like liver, brain, lungs, testes, kidneys, spleen, heart and pancreas (Osafanme, 2020). The biomarkers such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphate, GGT, alpha-amylase and dehydrogenase are used to study the possible toxicity in animals (Baker, 2021). The

administration of herbal drugs for 4 weeks in Swiss albino mice in both routes increased consistently compared to control groups.

Ordinarily, the liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been widely found in variety of tissues in body (Akannu, 2004). These enzymes are used as markers of hepatocellular injuries (liver injury or damage) or destruction of hepatocytes (Salasanti, 2014). In addition, increased AST, ALT and ALP (alkaline phosphate) indicates necrosis of hepatocytes or cytotoxic liver injury (Njagi, 2015). As the cells are damaged or injured, intracellular hepatic enzymes drain into blood circulatory system leading to increased serum levels in animals' body (Raza, 2002). These are the most sensitive biomarkers for liver cells damage. The liver cells damage results to overflow of enzymes to blood serum (Harris, 2005).

The increased amount of AST (in mitochondria) can be considered as symptom of cell injuries as a result of overflowing of enzymes into blood stream (Mdhuli, 2003). The ALT is a biochemical marker able to recognize hepatocellular damage than AST and helps confirm damage sustained by the liver cells (Al-mamary, 2002). The increased levels of ALT and AST in plasma indicate liver and kidney damages (Ogbonnia, 2008). The herbal drug caused increased amount of AST and ALT in blood plasma indicating that the dosage administered did not have hepato- and nephro- protective apparatus (Njagi, 2015). The increase in alkaline phosphate (ALP) amount is normally as a result of cholestasis or liver disease (Aniagu, 2005). The alkaline phosphate is a marker of cholestasis (Abiba, 2013). The finding from this study,

indicates that increase of ALP levels by the herbal extracts shows possible cholestasis occurred in the liver cells.

The increase of liver and kidney biochemical markers (ALP, LDH, GGT and alpha-amylase) indicated that herbal extracts doses administered affected cell integrities of treated experimental mice. The ALP enzyme speeds up the hydrolysis of inorganic phosphate preventing deposition of calcium to tissues (Yuji, 2013). The concentration of ALP enzyme is remarkably more in bone, kidney and liver (Neil, 2008). The increased levels of serum ALP (as a result of bile duct blockage) is associated with liver disease caused by intra or extra hepatic cholestasis (Baker, 2021l).

The LDH enzyme is involved in release of energy in muscles that facilitate the production of ATP through glycolysis of glycogen (the stored food in muscles) (Neil, 2008). The high secretion of LDH in blood is an alternative pathway to increase respiration (Raza, 2002). The high increase of LDH is also observed in individuals suffering from leukemia and liver diseases (Njagi, 2015). The increased levels of LDH indicates liver damages. The GGT mediates cells taking in extra-cellular glutathione (a component that is useful in anti-oxidation) (Neil, 2008). The glutathione is released during usual body biological functions and protects cells against oxidative stress (Subramanion, 2011). The GGT is also an indicator of abnormal hepatic functioning and biochemical marker of alcohol (Neil, 2008). The increased serum GGT amount in this study shows that hepatocellular damages occurred and was precipitated by phytochemicals possibly present in the plant extracts. The AST/ALT ratios measure the health status of the liver (Abiba, 2013).

The reduction of the AST/ALT ratios in this study indicates hepatic metastases and liver congestion in both administration routes.

The blood analytes such as urea, creatine and uric acid can be used to study functional capacity of liver and kidney (Laimeire, 2005). The results of blood analytes are good indicators of liver and kidney injury or damage (Baker, 2021). Findings from this work indicate that plant extracts caused significant dose dependent increase in creatine, urea and uric acid levels in both routes of administration. Urea and creatine are cellular protein metabolism wastes that need to be eliminated by the kidney (Abiba, 2013). Creatine is known as the good indicator of normal kidney functioning (Laimeire, 2005). From this study, the increased serum creatine levels suggest injury of kidney as a consequence of cytotoxic effects due to taking in of phytochemicals present in herbal extracts (Ugwu, 2013). The increase or decrease of blood urea nitrogen (BUN), normally corresponds with creatine (Abiba, 2013). This indicates that when creatine decreases, blood urea nitrogen also increases. The protein metabolisms or breakdown gives BUN as a final product. (Ugwu, 2013). This suggests that the herbal extracts administered might have directly damaged physiological integrities of cells in some organ like kidney (Osafanme, 2020). Uric acid is a final output of purines, constituents of nucleotides in deoxynucleic acids (Abiba, 2013). Most of uric acid (from DNA breakdown) is synthesized in liver and excreted through kidney during urination and defecation (Baker, 2021). The increased levels of UA as indicated from this study are as a result of liver and kidney atrophy due accumulation of urate. The increase of UA levels is also as a result of tissue breakdown (apoptosis) leading to body condition called hyperuricemia. (Abiba,

2013). Once uric acid crystals settle in joints (causes arthritis) and kidneys (causes kidney stones) (Ugwu, 2013).

Herbal drugs caused reduced volume of lipid indices and blood sugar/glucose. Tannins is associated with reduced appetite, indigestion, stomach upsets, and fat blockage and glucose malabsorption (Ugwu, 2013). This reduced concentration of triglycerides, cholesterol and glucose in blood system (Baker, 2021). Finding in this investigation, showed decreased lipid parameters and glucose in mice experimental models. The cytotoxic manifestation of the herbal drug could have interfered with breakdown and removal of wastes from liver hence decreased amounts of serum HDL-C, TG, LDL-C, TC and levels glucose (Abiba, 2013). The increased breakdown of glucose (glycolysis) is backed by low levels of sugar in both routes compared to the control experiment. Hence, close examination of glucose is essential especially for patients who are placed on this herbal therapeutics (Abiba).

The effects of heavy and trace metal found in the studied plant used to manage bacterial gastroenteritis is unknown. Only essential phyto-minerals are analyzed in this study and include calcium, potassium, iron, copper, chromium, mercury, vanadium, lead, magnesium, zinc, manganese, arsenic and strontium. Minerals in the plant extracts showed various concentration which is attributed to the differences in botanical structure, plant stress and soil elements where plant is growing (Ram, 2011). The other affecting factor is the preferential absorbability of certain minerals from the soil (Ram, 2011). Minerals are required for body to function properly but are only required in small amounts. That is, overdosing can upset body homeostasis feedback or mechanisms (Ram, 2011). The increase of doses of the herbal extracts implies

increased toxicity in animals. Some metal ions exposed to bacteria cause bacteria mortality such as Zinc ions (Miyano, 2010). This occurs through several mechanisms such as bacterial cell wall damage or rapturing of bacteria cell and breaking of the bacteria cellular components into pieces (Ram, 2011).

Metal such as Magnesium helps to maintain osmotic equilibrium and many enzymes catalyzed reactions (Abiba, 2013) and too much of it ca cause depression of central nervous system. Calcium is essential for healthy bones, muscles and nerves and its overdose can cause kidney stones (nephrolithiasis) (Ram, 2011). Excess copper causes violent outburst, exhaustion and causes stomach upsets (Kamar, 2005). Zinc is essential element, responsible for DNA/RNA synthesis (Ram, 2011).

Heavy metals such as lead, vanadium, arsenic and mercury are toxic once taken through ingestion and are lethal when in excess (Miyano, 2010). They produce damaging effects on respiratory organs (Ram 2011). Low levels of chromium maintain normal glucose metabolism (Ram, 2011). Chromium is a glucose tolerance factor (GTF) and also activates several enzymes (Abiba, 2013). Manganese is required in the body for various biochemical processes (Kamar, 2005). It also reduces nervous irritability (Ram, 2011). When Manganese is in excess can cause psychosis and hallucinations (Tarja, 2000). Findings from this study showed that all the minerals taken in treated animals were less than the required daily allowance in mice. Minerals such as Ca and Zn are reported to have antibacterial activity and should be incorporated into herbal anti-bacterial agents (Miyano, 2010). Minerals contained the extracts that help in restoring electrolyte and prevent rehydration of gastroenteritis patients (Abiba, 2013).

5.2 CONCLUSIONS

The findings from study indicate that;

1. Only *Zanthoxylum usambarense* methanol extracts demonstrated antibacterial activity using the Disk Diffusion Method, MIC, MBC, MBC/MIC values and Time-kill Assay against tested Gram-positive (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*, *Enterococcus feacalis*) and Gram-negative (*Escherichia coli*) bacteria. The inhibition is greater in Gram-positive than Gram-negative bacteria. Findings support continued use of plant extracts because it may be a good source of compounds with antimicrobial properties.
2. The methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* demonstrated toxicological effects as shown by changes in measured body weight, weight of organs, relative percentage of organ to body weight, haematological indexes and biochemical parameters.
3. The methanol plant extracts contained phyto-antimicrobial agents (alkaloids, flavonoids, terpenoids, tannins, glycosides) which may be responsible for antibacterial activity. Analysis of Z. *usambarense, Z. chalybeum* and *A. deserti* plants revealed presence of metals such as Na, K, Ca, Mg, Fe, Cu, Zn, Cr, Mn, Sr, As, V, Hg and Pb. The metal levels are in the extract are below the recommended daily allowance. This shows more free-radical activities hence more oxidation of lipids and high chances of damaging heart blood vessels.

5.3 RECOMMENDATIONS

From the results of this study and conclusions, the following actions are recommended to minimize risks of gastroenteritis in humans;

1) The *Zanthoxylum usambarense* plant extracts are highly recommended in the management of bacterial gastroenteritis. The optimal concentration for antibacterial activity was 2×MIC which demonstrated (99.9%) speed of killing susceptible microbes that causes gastroenteritis. The clinical study on these findings is recommended for commercial pharmaceutical formulations.

2) The methanol extracts of *Z. usambarense, Z. chalybeum* and *A. deserti* plants are reported in this study to contain chemical substances that increase lymphocytes suggesting that the extracts have immunostimulating properties. The herbal extracts are supported for their folkloric use or usage by traditional healers as they stimulate production of T-lymphocytes thereby boosting the defence system of the patients.

5.4 Suggestions for further studies

Further studies are needed in order to provide a broader picture on the management of gastroenteritis such as;

i) To determine intestinal anti-motility and diarrhoea reduction period of methanol extracts of *Zanthoxylum usambarense* to support the safe continued use plant in the management of gastroenteritis.

ii) To research on the mechanisms of antimicrobial activity of the plant extracts and the prescription of the medicinal agents in known dosages, to be used in rural communities where conventional drugs are unaffordable and inaccessible because of their high cost.

iii) To carry out toxicokinetics studies to establish the safety of *Z. usambarense, Z. chalybeum* and *A. deserti* plant extracts.

iv) To investigate the toxicity of the combined dosages and develop a rationalization of the combined therapeutics in management of bacterial gastroenteritis in humans.

v) To investigate the anti-microbial activity of the combined dosages. Besides, *Zanthoxylum chalybeum* and *Aloe deserti* may be effective while in combination with *Zanthoxylum usambarense* plant.

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APPENDICES

Appendix 1: Preparation of plant extracts

Experimental design: 450 mg/kg body weight, Swiss mice of weight of 23g needed, 10.35 mg of the plant extracts. There are 5 animals per group for each of the plant extracts which required 51.75mg. The extracts were administered for 28days which required 1449mg of the extracts. 670 mg/kg body weight needed, 15.41 mg of the herbal drug (5 animals required 2157.4mg of the extracts for 28days) while 1000 mg/kg body weight needed, 23 mg of the herbal drug (5 animals required 3220mg of the extracts for 28days).

Route of administration	5 animals per groups	Treatment (mg/kg bw)	Duration (days)
Oral	1 (0.1ml)	Saline	28
	2 (1449mg)	450	28
	3 (2157.4mg)	670	28
	4 (3220mg)	1000	28
Intraperitoneal	1 (0.1ml)	Saline	28
	2 (1449mg)	450	28
	3 (2157.4mg)	670	28
	4 (3220mg)	1000	28

This study used 8 groups of animals.

Appendix 2: McFarland Standard

For in vitro studies: Made by adding 0.05ml of 1% $Bacl_2$ to 9.95ml 0f 1% of H_2SO_4 and stored at 25^0C away from sunlight. The self-life 12 weeks.

McFarland	1%$Bacl_2$ (ml)	1% H_2SO_4 (ml)	Bacterial suspension/ml (turbidity)
0.5	0.05	9.96	1.5×10^8

Alternatively, bacterial suspension was adjusted to the same turbidity. Serial dilutions were made up to point 10^{-6} to verify the accuracy of McFarland standard by plate count methods. Preliminary studies indicated that;

i. 0.1ml of *Bacillus cereus* suspension plated at zero time from this point to 10^{-6} gave a growth of about 158, 150, 145 CFUs/plate at 0.5×MIC, MIC and 2×MIC respectively.

Dilution (Bacterial suspension)	**Number of colonies (From 0.1ml)**		
Concentrations	**0.5×MIC**	**1×MIC**	**2×MIC**
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	240	220
10^{-4}	250	180	160
10^{-5}	190	155	150
10^{-6}	158	150	145

ii. 0.1ml of *Bacillus subtilis* suspension plated at zero time from this point to 10^{-6} gave a growth of about 163, 150, 148 CFUs/plate at 0.5×MIC, MIC and 2×MIC respectively.

Dilution (Bacterial suspension)	Number of colonies (From 0.1ml)		
Concentrations	**0.5×MIC**	**1×MIC**	**2×MIC**
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	250	260
10^{-4}	260	180	160
10^{-5}	200	155	150
10^{-6}	163	150	148

iii. 0.1ml of *Staphylococcus aureus* suspension plated at zero time from this point to 10^{-6} gave a growth of about 182, 158, 152 CFUs/plate at 0.5×MIC, MIC and 2×MIC respectively.

Dilution (Bacterial suspension)	Number of colonies (From 0.1ml)		
Concentrations	**0.5×MIC**	**1×MIC**	**2×MIC**
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	260	250
10^{-4}	280	220	180
10^{-5}	200	165	160
10^{-6}	182	158	152

iv. 0.1ml of *Enterococcus faecalis* suspension plated at zero time from this point to 10^{-6} gave a growth of about 190, 168, 158 CFUs/plate at 0.5×MIC, MIC and 2×MIC respectively.

Dilution (Bacterial suspension)	Number of colonies (From 0.1ml)		
Concentrations	**0.5×MIC**	**1×MIC**	**2×MIC**
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	280	270
10^{-4}	290	240	200
10^{-5}	220	195	170
10^{-6}	190	168	158

v. 0.1ml of *Escherichia coli* suspension plated at zero time from this point to 10^{-6} gave a growth of about 250, 199, 180 CFUs/plate at 0.5×MIC, MIC and 2×MIC respectively.

Dilution (Bacterial suspension)	Number of colonies (From 0.1ml)		
Concentrations	**0.5×MIC**	**1×MIC**	**2×MIC**
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	280	270
10^{-4}	290	240	220
10^{-5}	260	210	180
10^{-6}	250	199	158

Appendix 3: Instrumental conditions for metal analysis (AAS)

Metal	Ca	Zn	Cu	Mg	Cr	Pb
Wavelength(nm)	422.7	213.9	324.8	285.2	357.9	217
Current (mA)	3.5	5.0	5.0	5.0	5.0	10
Shift width (nm)	0.2	0.2	0.2	0.5	0.2	1
Flow rate						
Air	7.0	7.0	7.0	7.0	7.0	7.0
Acetylene	1.8	1.8	1.8	1.8	1.8	1.8

(Ram, 2011)

Appendix 4: Hematology. Normal Reference Values

RBC ($\times 10^{-6}$µl)	6.36 – 9.42
Hb (g/dL)	11.0 – 15.1
PCV (%)	35.1 – 45.4
MCH (g/dL)	14.1 – 19.3
MCHC (g/dL)	30.2 – 34.2
MCV (fL)	45.4 – 60.3
WBC ($\times 10^{-3}$/l)	1.8 – 10.7
Lymphocytes (%)	55.8 – 91.6
Neutrophils (%)	6.6 – 38.9
Monocytes (%)	0.0 – 7.5
Eosinophils (%)	1.0 – 3.0
Basophils (%)	0.0 – 0.1

(CDC, 2018)

Appendix 5: Specimens voucher information

Identification Botanical name	Kimeru name	Collection location	Plant parts collected	Voucher number
Aloe deserti	Chukurai	Rwarera	Whole plant	SM01
Zanthoxylum chalybeum	Muluiri	Lairang'i	Twigs	SM02
Zanthoxylum usambarense	Mubuchwa	Laare	twigs	SM03