IN VITRO ANTIBACTERIAL, *IN VIVO* IMMUNOMODULATORY AND SAFETY PROPERTIES OF ETHYL ACETATE LEAF EXTRACT OF *Ocimum basilicum*

WAMBANI JAPHETH RAPANDO (BSc., MSc.)

P97/33760/2015

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MEDICAL MICROBIOLOGY) IN THE SCHOOL OF MEDICINE OF KENYATTA UNIVERSITY

NOVEMBER, 2021

DECLARATION

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

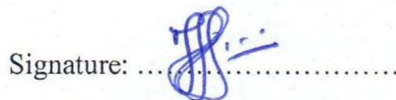
Signature: Date: 11.11.2021

Japheth Wambani Rapando; Reg. No. P97/33760/2015

Department of Pathology, Kenyatta University

Supervisors

This thesis has been submitted for examination with our approvals as the University Supervisors.

Signature:  Date: 15/11/2021

Dr. James Ogutu

Department of Medical Microbiology and Parasitology

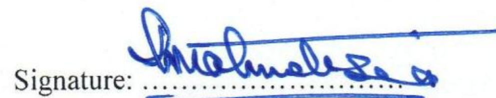
Kenyatta University

Signature: Date: 15.11.2021

Dr. Margaret Muturi

Department of Medical Laboratory Sciences

Kenyatta University

Signature:  Date: 15/11/2021

Dr. Mathew Piero Ngugi

Department of Biochemistry, Microbiology and Biotechnology

Kenyatta University

DEDICATION

To Kimberley Mideva Rapando

ACKNOWLEDGEMENTS

Thanks be to God for the gift of life and for the strength He has bestowed upon us from which this endeavor was accomplished. I'm fortunate to know so many smart and capable people. Without them, this thesis wouldn't be in your hands. I would like to express my sincere gratitudes to my supervisors Dr. James Ogutu whose criticisms and guidance have been invaluable and Dr. Margaret Muturi for her encouragement. To Dr. Mathew Piero Ngugi, who has been a tremendous mentor, thank you for encouraging my research and for allowing me to grow as a research scientist. You have all been incredibly patient in guiding this thesis from its crude early stages to the polished product that it is.

My sincere thanks also go to Dr. Scholastica Mathenge, Prof. Steven Runo and Dr. Gladys Mwangi, who provided me with an opportunity to access departmental laboratories and research facilities. Without their precious support, it would have been tough conducting this research. Dr. Anthony Ireri 'Cewa', thank you for the scholarship, and long may it continue!

I would like to thank my family; my parents Maurice Wambani Rapando and Julia Jamhuri Aswani, my brother Victor Rapando Aswani, and sisters Margaret Auma Rapando, Mercy Rapando and Daisy Rapando, my niece Cynthia Akoth, daughter Kimberley Mideva Rapando, uncle Lawrence Aswani and aunt Jane Aswani. You all have ensured that I have not lacked spiritual, moral and emotional support through my studies. To my parents, thank you for inculcating within us, the need to achieve academic

axcellence and for all the sacrifices you have made for us to reach this far. My forever encouraging, interested and always enthusiastic grandmother Maria Adipo Wambani: You are always keen to know what I am doing and how I am proceeding, although it is more likely that you have never grasped what it is all about! I hope you still have the voice to scream for when a significant momentous is reached.

Many thanks to the laboratory technicians in the departments of Biochemistry, Microbiology and Biotechnology, Medical Laboratory Sciences and Pharmacy, Complementary and Alternative Medicine whose guidance was immense during my research. Thank you to Daniel Gitonga, James Ngunjiri, Barbra and Joyce for the technical support during my project work. Gratitude to Mr. James Adino for showing me the finer details of handling animals and for answering my odd questions without blinking!

I thank my fellow lab mates and colleagues: Dr. Wycliffe Arika, Wilhelmy Jebet, Dr. Dorothy Wavinya, Christine Nakhumicha, James Kimani, Jonathan Mateba, Philip Ogola, Hibert Rachuonyo, Shadrack Njagi, Peterson Mucheni, Stephen Gitahi, Stephen Mwihia, Kelvin Juma, Harry Musonye and all that made this work possible – it's impossible to mention all of you, you wouldn't fit into this thesis! I have had so many academic colleagues in my entire educational journey but I have never encountered a group so bound to their academic ideologies and just damned friendly!

With gratitude, and may The Almighty bless all of you, in abundance.

TABLE OF CONTENTS

DECLARATION .... **II**

DEDICATION .... **III**

ACKNOWLEDGEMENTS .... **IV**

TABLE OF CONTENTS .... **VI**

LIST OF TABLES .... **XI**

LIST OF FIGURES .... **XII**

ACRONYMS AND ABREVIATIONS .... **XIII**

ABSTRACT .... **XVI**

CHAPTER ONE **1**

1.0 INTRODUCTION **1**

1.1 Background 1
1.2 Statement of the Problem 6
1.3 Justification of the Study 7
1.4 Research Questions 8
1.5 Hypotheses 8
1.6 Objectives 9
1.6.1 General Objective 9
1.6.2 Specific Objectives 9

CHAPTER TWO **10**

2.0 LITERATURE REVIEW **10**

2.1 Bacteriology of Nosocomial Pathogens 10
2.1.1 *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* .. 11
2.1.2 *Pseudomonas aeruginosa* 13
2.1.3 *Escherichia coli* 14
2.2 Risk Factors for Transmission of Nosocomial Infections 15
2.2.1 Mechanical Ventilators 15
2.2.2 The Intensive Care Unit 16
2.2.3 Age 16
2.2.4 Antibiotic Misuse and Biofilm Formation 17
2.2.5 Other factors 17
2.3 Epidemiology of Nosocomial Infections 18
2.4 The Immunobiology of Bacterial Pathogens 20
2.4.1 The Innate Immune Responses 21

2.4.1.1 Tumor Necrosis Factor-alpha 22
2.4.1.2 Interleukin 1 22
2.4.1.3 Interleukin-10 23
2.4.1.4 Interleukin 17 24
2.4.1.5 Polymorphonuclear Neutrophils 25
2.4.2 Adaptive Immune Responses 27
2.5 Antibiotics and Antibiotic Classes 30
2.5.1 Antibiotic Toxicity and Adverse Effects 31
2.5.2 Antibiotic Resistance Mechanisms 32
2.5.2.1 Penicillin and Methicillin Resistance 33
2.5.2.2 Extended Spectrum beta-Lactamases 34
2.5.2.3 Carbapenemases 35
2.5.2.4 Vancomycin Resistance 35
2.6 Control and Prevention of Nosocomial Pathogens 36
2.7 *Ocimum basilicum* and Herbal Management of Infectious Diseases 38
2.7.1 Phytochemicals and Antimicrobial Mechanisms 41
2.7.1.1 Flavonoids 42
2.7.1.2 Alkaloids 42
2.7.1.3 Phenols, Polyphenols and Tannins 43
2.7.1.4 Quinones 44
2.7.1.5 Saponins 44
2.7.1.6 Glycosides 44
2.7.1.7 Terpenoids and Essential Oils 45
2.7.1.8 Lectins and Polypeptides 45
2.8 Immunomodulatory Effects of Plant Extracts 45
2.9 Mineral elements and Heavy Metals 47
2.10 Safety of Plant Extracts as Therapeutic Agents 52
2.11 Biochemical and Hematological Markers of Toxicity 54
2.11.1 Biochemical Markers 54
2.11.1.1 Liver Biomarkers 54
2.11.1.2 Kidney Biomarkers 56
2.11.1.2.1 Creatinine 56
2.11.1.2.2 Urea 57
2.11.1.3 Hematological Markers 57
2.11.1.3.1 Red Blood Cells and Related Indices 57
2.11.1.3.2 White Blood Cells and Related Indices 58
2.11.1.3.3 Platelets (PLT) 58

CHAPTER THREE 60

3.0 MATERIALS AND METHODS 60
3.1 Collection of *O. basilicum* 60
3.2 Processing and Extraction 61
3.3 Spectrophotometric Methods 62
3.3.1 Quantitative Phytochemical Analysis 62
3.3.2 Quantitative Mineral Element and Heavy Metal Analysis 63
3.4 *In vitro* Antibacterial Assays 64
3.4.1 Extract Dosage Preparation for Antibacterial Assays 64
3.4.2 Bacterial Strains 64
3.4.3 Inoculum Preparation and Antibacterial Assays 65
3.4.3.1 Determination of Minimum Inhibitory Concentrations (MIC) 66
3.4.3.2 Determination of Minimum Bactericidal Concentration 67
3.5 Determination of *In vivo* Immunomodulatory Activities 68
3.5.1 Drugs 68
3.5.2 Antigen Preparation 68
3.5.3 Experimental Animals 69
3.5.4 Experimental Designs for the Immunomodulatory Effects of the Ethyl acetate Leaf Extract of O. basilicum 70
3.5.4.1 Determination of the Effect of the Ethyl Acetate Leaf Extract of *O. basilicum* on Cyclophosphamide Induced Myelosuppression 70
3.5.4.2 Determination of Hemagglutinin Antibody Titers and Neutrophil Adhesion Tests 73
3.5.4.3: Determination of RRBC - Induced Delayed Type Hypersensitivity (DTH) Responses, WBC and Platelet Counts 77
3.5.4.4 Determination of Carbon Clearance for Phagocytic Activity 80
3.6 28-Day Repeated-dose Oral Toxicity Study in Mice 82
3.6.1 Experimental Mice 82
3.6.2 Experimental Design 83
3.6.2.1 Blood Collection 84
3.6.2.2 Hematological Assays 84
3.6.2.3 Biochemical Assays 84
3.6.2.4 Absolute Organ Weights and Organ Indices 85
3.7 Ethical Approval 85
3.8 Data Analysis 85

CHAPTER FOUR 87

4.0 RESULTS 87
4.1 Percentage Yield 87

4.2 Phytochemical Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* 87
4.3 Mineral Elements Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* 92
4.4 Heavy Metal Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* . 93
4.5 *In vitro* Antibacterial Activity of the Ethyl Acetate Leaf Extract of *O. basilicum* 93
4.5.1: Antibacterial Activity on the *S. aureus* isolate and quality control strain ... 96
4.5.2 Antibacterial Activity of the extract on the clinical isolate of *E. coli* and the quality control strain 98
4.5.3 Antibacterial Activity on *P. aeruginosa* 98
4.5.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration 101
4.6 Immunomodulatory Potential of Ethyl acetate Leaf Extract of *O. basilicum* ... 104
4.6.1 Effect of *O. basilicum* Extract on Cyclophosphamide Induced Myelosuppression 104
4.6.1.1: Effect of the Extract on Hb levels after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice 107
4.6.1.2: Effect of the Extract on Total Leucocyte Counts after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice..... 108
4.6.1.3: Effect of the Extract on Red Blood Cell Count after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice........ 110
4.6.2 Effect of the Extract on Humoral Antibody Responses to RRBC's in Swiss Albino Mice 111
4.6.3: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Neutrophil Adhesion in Swiss Albino Mice 113
4.6.4: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Mean Foot Paw Edema in DTH Models 115
4.6.4.1: Effects of Ethyl acetate extract of *O. basilicum* on Total Leucocyte and Platelet Counts in DTH Models 118
4.6.6: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Carbon Clearance Test for Phagocytic Activity in Swiss Albino Mice 122
4.7 *In vivo* Safety Evaluation of the Ethyl acetate Extract of *O. basilicum* in Normal Swiss albino Mice Models 124
4.7.1 Effect on Observatory and Behavioral Indices 124
4.7.2 Effect of the Ethyl acetate Extract of *O. basilicum* on Absolute Body Weights 125

4.7.3 Effect of the Ethyl acetate Extract of *O. basilicum* on Absolute Organ Weights 126
4.7.4 Effect of the Ethyl acetate Extract of *O. basilicum* on the Relative Organ Weight to Body Weight 128
4.7.5 Effect of the Ethyl acetate Extract of *O. basilicum* on Hematological Parameters 129
4.7.5.1 Red Blood Cell Indices 129
4.7.5.2 White Blood Cell Indices 130
4.7.5.3 Platelet Indices 131
4.7.6 Biochemical Parameters 132
4.7.6.1 Liver Function Markers 132
4.7.6.2 Renal Function Markers 134

CHAPTER FIVE 135

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 135
5.1 Discussion 135
5.1.1 Phytochemical Composition of the Ethyl acetate Extract of *O. basilicum* . 135
5.1.2 Essential and Trace Mineral Element Contents of Ethyl acetate Extract of *O. basilicum* 141
5.1.3 Antibacterial Activity of the Ethyl Acetate Leaf Extract of *O. basilicum* .. 147
5.1.4 Immune Modulation Effects of Ethyl Acetate Leaf Extract of *O. basilicum* 152
5.1.5 *In vivo* Safety Profile of the Ethyl Acetate Leaf Extract of *O. basilicum* ... 160
5.2 Conclusions 167
5.3 Recommendations 168
5.4 Suggestions for Further Studies 168

REFERENCES 169

APPENDICES 210

LIST OF TABLES

Table 3.1: Experimental Design for the Determination of the Effect of Ethyl Acetate Leaf Extract of *O. basilicum* on Cyclophosphamide Induced Myelosuppression 72
Table 3.2: Experimental Design for Determination of Hemagglutinin Antibody Titer and Neutrophil Adhesion Tests 75
Table 3.3: Experimental Design for RRBC - Induced Delayed Type Hypersensitivity Responses, WBC and Platelet Counts 79
Table 3.4: Experimental Design for Carbon Clearance for Phagocytic Activity 81
Table 3.5: Experimental Design for Safety Evaluation 83
Table 4.1: Phytochemical Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* 88
Table 4.2: Mineral Elements Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* 92
Table 4.3: Heavy Metal Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum* 93
Table 4.4: An Antibiogram of Bacterial Strains 95

LIST OF FIGURES

Figure 2.1: Sweet basil (*Ocimum basilicum*) (Source: Authors). 41
Figure 3.1: Map showing Nthawa location, Embu County from where the plant was collected.. 61
Figure 3.2: The Swiss Albino Mice (Source: Authors) 69
Figure 4.1: A chromatogram of the ethyl aceate leaf extract of *O. basilicum*.................. 91
Figure 4.2: Zones of inhibition of Gentamycin and different concentrations of the plant extracts on MRSA…………………………………………………………..92
Figure 4.3a: Zones of inhibition on the ATCC strain of *S. aureus*………………………94
Figure 4.3b: Zones of inhibition of plant extracts on the clinical isolate of *S. aureus* 97
Figure 4.4a: Zones of inhibition on the ATCC strain of *P. aeruginosa.* 99
Figure 4.4b: Zones of inhibition on the clinical isolate of *P. aeruginosa* 100
Figure 4.5: A 96 micro-well plate demonstrating MIC value determination 102
Figure 4.6: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Humoral Antibody Responses to RRBC. Bars with the same letter are not significantly different. 112

ACRONYMS AND ABREVIATIONS

AdS	Adenosine synthase A
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of Variance
As	Arsenic
AST	Aspartate transaminase
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Ba	Barium
Ca	Calcium
CA-MRSA	Community associated methicillin resistant *Staphylococcus aureus*
Cd	Cadmium
CD	Cluster of Differentiation
CFU	Colony forming units
Cl	Chlorine
CLSI	Clinical Laboratories Standards Institute
Cr	Chromium
CRE	Creatinine
Cu	Copper
CXCR2	Chemokine receptor 2
DBIL	Direct bilirubin
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DTH	Delayed type hypersensitivities
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra-acetic acid
EHEC	Enterohemorrhagic *E. coli*
ELISA	Enzyme linked Immunosorbent Assay
EPEC	Enteropathogenic *E. coli*
ESBLs	Extended-spectrum β -lactamases
Fe	Iron
GR	Granulocytes
HA	Hemaglutinnin
HCT	Hematocrit
Hg	Mercury
Hb	Hemoglobin
HLA-DR	Human Leucocyte Antigen – Antigen D related
HlyA	α-haemolysin
HNP	Human neutrophil peptides
ICU	Intensive care unit
IFN-γ	Interferon-gamma

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-17	Interleukin 17
IL-1R	Interleukin 1 receptor
IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-2	Interleukin 2
IL-22	Interleukin 22
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IOM	Institute of Medicine
K	Potassium
LY	Lymphocytes
MBC	Minimum bactericidal concentration
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Mg	Magnesium
MHA	Mueller Hinton Agar
MIC	Minimum inhibitory concentration
Mn	Manganese
MO	Monocytes
MPV	Mean Platelet Volume
mRNA	messenger RNA
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
Na	Sodium
ND	Not detected
Ni	Nickel
NLR	Nod-like receptors
O. basilicum	*Ocimum basilicum*
O. canum	*Ocimum canum*
O. gratissimum	*Ocimum gratissimum*
O. sanctum	*Ocimum sanctum*
O. tenuiflorum	*Ocimum tenuiflorum*
P	Phosphorous
P-LCR	Platelet large cell ratio
P. aeruginosa	*Pseudomonas aeruginosa*
PAMPs	Pathogen associated molecular patterns
Pb	Lead

PBP	Penicillin Binding Protein
PCR	Polymerase chain reaction
PCT	Plateletcrit
PDW	Platelet Distribution Width
PLT	Platelets
PRRs	Pattern Recognition Receptors
RBC	Red Blood Cells
RDW	Red Blood Cell Distribution Width
RNA	Ribonucleic acid
RRBC	Rabbit red blood cell
rRNA	ribosomal Ribonucleic Acid
S	Sulphur
S. aureus	*Staphylococcus aureus*
S.D	Standard deviation
Sbi	staphylococcal binder of immunoglobulin
SCCmec	staphylococcal chromosome cassette mec
Se	Selenium
SpA	Staphylococcal protein A
SSI	Surgical site infections
STAT3	Signal transducer and activator of transcription 3
TBIL	Total bilirubin
TC	Total Cholesterol
TG	Triglycerides
Th	T helper
TLC	Total leucocyte counts
TLR	Toll like receptors
TNF-α	Tumor necrosis factor alpha
TP	Total Protein
U.S	United States
UTI	Urinary tract infections
VAP	Ventilator associated pneumonia
VISA	Vancomycin intermediate *S. aureus*
VRSA	Vancomycin Resistant *S. aureus*
WHO	World Health Organization
Zn	Zinc

ABSTRACT

Staphylococcus aureus (S. aureus), Methicillin resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli (E. coli)* are common bacterial pathogens. Multi-drug resistant strains of these pathogens have led to a demand for new treatment options. *O. basilicum* is an important medicinal plant and has traditionally been shown to have antimcrobial values. However, there was need to validate its safety and immune modulation potential. The aim of this study was to determine the *in vivo* immunomodulatory, safety and *in vitro* antibacterial effects of *O. basilicum*. This was a laboratory-based experimental study. *Ocimum basilicum* was obtained from Siakago, Embu County. Different concentrations of the ethyl acetate leaf extract were used for testing antibacterial efficacies by the disc diffusion method followed by determination of MIC and MBC by broth dilution. Phytochemical and elemental properties of the extract were assayed by GC-MS and atomic absorbance spectrophotometry. For immune modulation, 300, 200 and 100 mg/kgbw extract concentrations were used while RRBC's were used as the antigen. Administration of mice with 300, 548 and 1000 mg/kgbw for 28 days was done to determine their body weight, organ weight, biochemical and hematological profiles for safety evaluation. Ethical approval for the use of mice was sought from the Kenyatta University animal care and use committee. Phytochemical composition of the extract included terpenoids, alkaloids, essential oils, fatty acids, flavonoids and aldehydes. Of the mineral elements detected in the extract, Mg had the highest concentration (1241.6±0.42 mg/100g) while cobalt had the lowest concentration (0.08±0.02 mg/100g). Heavy metals were not detected. A broad spectrum antibacterial activity was exhibited on the tested bacterial strains. The highest activity was on *P. aeruginosa* (27.00±2.00mm) while the lowest activity was on the isolate of *S. aureus* (17.33+0.58mm). The extract reversed the effects of cyclophosphamide on the myeloid stem cells by restoring the counts of Hb, WBC and RBC's in the blood. There was a dose dependent effect on the humoral antibody responses with the 300 mg/kgbw concentration of the extract exhibiting statisticaslly significant (12.4±0.894) antibody responses on the RRBC's. The extract exhibited an effect on neutrophil adhesion and a dose dependent effect on phagocytic index which wasn't significantly different among the extract concentrations. In DTH models, the different extract concentrations had dose dependent effects on TLC's and PLT counts. *In vivo* safety tests revealed that the 1000 mg/kgbw concentration significantly lowered PLT counts (556.00±76.00 10^3/µl) while having no effect on other blood indices. The extract was also proven safe on the liver and kidney. The ethyl acetate leaf extract of *O. basilicum* had antibacterial, immune modulation effects and was safe for *in vivo* use, therefore, this plant could be harnessed in efforts to come up with alternative and complementary therapies.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus*), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) are among the most common causes of hospital associated infections (Marra *et al.,* 2011). *Staphylococcus aureus* is a Gram-positive bacterium commonly isolated from nosocomial samples and is the second most common pathogen isolated from outpatients (Kuehnert *et al.,* 2005). It forms part of the normal flora of the upper respiratory tract with about 25% of the human population being chronic healthy carriers (Belkum *et al.,* 2009).

Changes in the susceptibility of *S. aureus* to beta-lactams have been widely reported. Methicillin resistant *S. aureus* emerged due to different resistance mechanisms including production of penicillin-binding protein (PBP) that possesses a low affinity for β-lactam antibiotics and secretion of the penicillinase enzyme that breaks down the β-lactam ring, which is responsible for antimicrobial action (Anand *et al.,* 2009; Dilnessa and Bitew, 2016). Methicillin resistant *S. aureus* is difficult to eradicate and spreads more rapidly than other *S. aureus* strains, accounting for up to 75 % of all *S. aureus* isolates in some countries (Dilnessa and Bitew, 2016).

Escherichia coli is a Gram-negative bacterium occurring as a normal intestinal flora in the human body. However, *E. coli* can become harmful once there is a slight genetic recombination that brings about a pathogenic strain capable of causing a variety of infections that include sepsis, meningitis, traveler's diarrhea, pneumonia and bacteremia (Kim *et al.,* 2013). It is the most common agent involved in the causation of Urinary Tract Infections (UTIs) in hospital and community settings (Mamuye, 2016).

The Gram-negative opportunistic bacterium, *P. aeruginosa,* is a pathogen that inhabits the soil and surfaces of aqueous environments (Gellatly and Hancock, 2013; Wonnenberg *et al.,* 2016). Its high antibiotic resistance mechanisms and adaptability enable it to survive in a range of both artificial and natural settings (Gellatly and Hancock, 2013). Nearly all infections associated with *P. aeruginosa* occur in immune-compromised individuals and is a major cause of respiratory infections among hospitalized patients (Wonnenberg *et al.,* 2016).

Nosocomial infections are a major concern worldwide, contributing to morbidity, mortality and overall healthcare costs. The magnitude and scope of nosocomial infections are immense and, therefore, challenging to enumerate with accuracy (Llata *et al.,* 2009). In the United States (U.S) and other high income countries such as France, Australia, Japan and Germany, hospitalized individuals who got infected with at least one nosocomial pathogen in 2011 ranged from 3.5% to 12% (WHO, 2011). Over 4 million and 1.7 million individuals are affected by nosocomial pathogens every year in Europe

and the U.S respectively with the majority of the infections occurring among the Intensive Care Unit (ICU) patients (WHO, 2011).

Studies done among ICU patients in France, Italy and Germany revealed that *S. aureus* (21.8%), *Pseudomonas* spp. (17.2%), enterobacteriaceae (20.2%), enterococci (10.0%), *Candida* spp (8.8%), *E. coli* (9.1%), *Acinetobacter* spp (5.1%) and coagulase-negative staphylococci (7.0%) were the most common causes of infections (Legras *et al.,* 1998; Gastmeier *et al.,* 2007; De Rosa *et al.,* 2008; Malacarne *et al.,* 2010).

There is paucity of data on nosocomial infections and drug resistance in developing nations (Okeke *et al.,* 2005; WHO, 2011). Allegranzi *et al.* (2011) reported that Gram positive rods are the most common isolates in nosocomial samples with *S. aureus* being the most common and *Acinetobacter* spp. being the second most frequent at 24%. As many as 54.5% of all *S. aureus* clinical isolates showed resistance to methicillin (WHO, 2011).

In Kenya, incidences of wound infections after delivery were documented at 19% in Central Kenya (Koigi-Kamau *et al.,* 2005). In Kilifi, incidences of bacteremia due to *S. aureus* in children of less than five years of age was 27% (Berkley *et al.,* 2005). Aiken *et al.* (2014) reported a 7% incidence of MRSA in Thika level five hospital. With these prevalences, there is need to develop new control and treatment options.

Formation of neutrophil abscesses in extracellular infections is required for pathogen clearance. Neutrophils are the key effector cells in the orchestration of adaptive immunity

and resolution of inflammatory responses (Molne *et al.,* 2000; Jaillon *et al.,* 2013). Neutrophil recruitment to sites of infection involve bacterial recognition by pattern recognition receptors (PRRs) such as nucleotide-binding oligomerization domain proteins and toll like receptors (TLR) as well as the initiation of pro-inflammatory responses (Molne *et al.,* 2000). Neutrophil recruitment is mediated by T helper 1 (Th1) inflammatory cytokines, which include Interferon-gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), T helper 2 (Th2) inflammatory cytokines including Interleukin 6 (IL-6), T helper 17 (Th17) inflammatory cytokine, Interleukin 17 (IL-17), interleukin 1-alpha (IL-1α), interleukin 1-beta (IL-1β), and chemokines that mediate neutrophil chemo-attraction through the CXC-chemokine receptor 2 expressed on neutrophils (Kawai and Akira, 2010; Jaillon *et al.,* 2013).

Interferon gamma secreted by Th1 promotes cell mediated immunological responses, Th2 cells secrete IL-4 that promotes antibody-mediated immune responses, while IL-17 secreted by Th17 cells, promote neutrophil chemo-attraction and abscess formation (O'shea and Paul, 2010). Interleukin 10 (IL-10) and Interleukin 4 have been expressed during *S. aureus* persistence in the body and have been attributed to aid in bacterial survival (Chau *et al.,* 2009; Peres *et al.,* 2015; Heim *et al.,* 2015). Finding alternative ways of upregulating immune responses against these pathogens is important for pathogen clearance and could offer new perspectives on immunotherapy.

These pathogens are highly resistant to conventional antibiotics. Antibiotic resistance is a growing concern. Mechanisms of resistance vary and sometimes resistance can occur to

multiple classes of antibiotics. Resistance to beta-lactam antibiotics, carbapenems and other antimicrobial classes has been reported (Rafailidis, 2010; Mamuye, 2016; DeFrancesco *et al.,* 2017; Ma *et al.,* 2017; Dias *et al.,* 2017).

Due to antibiotic resistance, efforts to find new therapeutic agents have led to an increase in research on plants with antimicrobial and immune stimulation potential. In survival, plants are a man's best friend. They have provided fuel, food and medicine since the days beyond the dawn of civilization. As they have been throughout human history, they continue to be a major source of medicine (Devendran and Balasubramanian, 2011). *Ocimum basilicum* (*O. basilicum*) is traditionally used to treat many infections and has been particularly shown to have antimicrobial action on many pathogens (Kaya *et al.,* 2008; Hanif *et al.,* 2010; Dambolena *et al.,* 2010; Runyoro *et al.,* 2010; Yahya, 2011; Joshi, 2014). Its leaves have been shown to treat wounds in animal models (Yahya, 2011).

Phytochemicals such as flavonoids, tannins, saponins, cardiac glycosides have been reported to be present in different species of *Ocimum* with accompanying antibacterial, antiviral and antiparasistic effects (Akiyama *et al.,* 2001; Bajaj, 2005; Devendran and Balasubramanian, 2011). Mineral elements and trace elements found in plants include Potassium (K), Manganese (Mn), Sodium (Na), Calcium (Ca), Barium (Ba), Iron (Fe), Zinc (Zn), Copper (Cu), Phosphorous (P), Selenium (Se), Nickel (Ni), Sulphur (S), Chromium (Cr) and Magnesium (Mg). They are nutritionally important as they aid in bone formation, enzyme activities and alleviation of the toxic effects of heavy metals

(Nazar *et al.,* 2012; Fagbohun, *et al.,* 2012; Mahlangeni, *et al.,* 2014). Trace elements serve as enzymatic co-factors thereby playing important roles in metabolism (Kumar *et al.,* 2017). Elemental composition of plants is needed to assess their food, medicinal values and immunomodulatory effects.

Heavy metals such as Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) are absorbed by plants from the soil and are detrimental for the plants, humans and animals (Kumar *et al.,* 2017). Mineral elements and heavy metals compete for the same transport system, therefore, inhibiting the uptake and transportation of important elements (Nazar *et al.,* 2012). Heavy metals have a bearing on the safety of medicinal plants *in in* vivo uses.

This study, therefore, aimed at ascertaining the immune modulation and antibacterial effects of the ethyl acetate leaf extract of *O. basilicum*. The phytochemical, mineral elements and heavy metal composition of the extract as well as its *in vivo* safety were also determined.

1.2 Statement of the Problem

Infectious diseases are the leading cause of morbidity and mortality worldwide. Of global importance is the rise in antibiotic resistance with the clinical efficacy of a majority of the available antibacterials being threatened by emerging multidrug resistant pathogens. The rising prevalence of antibiotic resistant infections caused by bacteria such as *S. aureus, P. aeruginosa*, MRSA and *E. coli* with the formation of new strains, which have a reduced predisposition to antibiotics has raised the specter of untreatable infections and added to

the urgent need for new antibacterial strategies. *Staphylococcus aureus, P. aeruginosa*, MRSA and *E. coli* are pathogens of ever increasing importance both in the hospital and community settings. Due to antibiotic resistance, treatment combinations of antibacterial agents have been used. However, reports of nephrotoxicity and other adverse effects have been documented relating to drug combinations in treating infections. Majority of these drugs are also not easily accessible. There is paucity of data on traditional medicine with promising potential to manage these infections. The perception that herbs, being naturally used worldwide, are safe for medicinal purposes is not supported by adequate scientific data. No scientific rationale exists to ascertain that *O. basilicum*, its derived products or parts are safe for consumption among the Mbeere community compared to the conventional medicines, its popular use notwithstanding. The heavy metal content, trace element and phytochemical composition of this plant has not been established despite the fact that they have a bearing on the antibacterial and immunomodulatoryu effects.

1.3 Justification of the Study

In spite of the existing potent antibacterial agents, resistant and multidrug resistant strains are always evolving. This has increased the need for research and development of new antibacterials which are safe, more dependable, less in toxicity and cost than the existing conventional drugs. Medicinal plants such as *O. basilicum* have been and continue to be the best source of medicines. The advantages put forward for the use of plants for therapeutic purposes stem from the fact that they are safe, economical, easily available and effective. Biomedical researchers, in their pursuit of new treatment options for infectious diseases, are drawing their efforts towards folk medicine. Phytochemicals and

oils, which are secondary metabolites in plants are potential drug sources of therapeutic importance. Essential mineral elements and heavy metals were determined in this medicinal plant for the assessment of its medicinal values and toxicological potential. Evaluation of infectious disease interventions that target the hosts' immune responses reduce the challenges associated with bacterial drug resistance while opening up new avenues for immunotherapy. This study therefore aimed at ascertaining the immune modulation and antimicrobial effects of the ethyl acetate leaf extract of *O. basilicum* as well as its phytochemical, mineral elements and heavy metal composition. The *in vivo* safety of the extract was also determined.

1.4 Research Questions

i. What are the proximate levels of phytochemicals, mineral elements and heavy metals in the ethyl acetate leaf extract of *O. basilicum*?

ii. Does the ethyl acetate leaf extract of *O. basilicum* have any *in vitro* antibacterial activities on *S. aureus*, MRSA, *P. aeruginosa* and *E. coli*?

iii. What is the immunomodulatory potential of the ethyl acetate leaf extract of *O. basilicum* in normal Swiss albino mice?

iv. What is the *in vivo* safety of the ethyl acetate leaf extract of *O. basilicum* in normal Swiss albino mice?

1.5 Hypotheses

i. Ho1: There are no phytochemicals, mineral elements and heavy metals in the ethyl acetate leaf extract of *O. basilicum*.

ii. Ho2: The ethyl acetate leaf extract of *O. basilicum* does not have any antibacterial activity on *S. aureus*, MRSA, *P. aeruginosa* and *E. coli in vitro*.

iii. Ho3: The ethyl acetate leaf extract of *O. basilicum* does not have immune modulatory effects in Swiss white albino mice.

iv. Ho4: The ethyl acetate leaf extract of *O. basilicum* is not safe for therapeutic use in Swiss white albino mice.

1.6 Objectives

1.6.1 General Objective

To determine the *in vitro* antibacterial, *in vivo* immune modulation and safety of the ethyl acetate leaf extract of *O. basilicum.*

1.6.2 Specific Objectives

i. To determine the proximate levels of phytochemical, mineral elements and heavy metal in the ethyl acetate leaf extract of *O. basilicum.*

ii. To determine the *in vitro* antibacterial activities of the ethyl acetate leaf extract of *O. basilicum* against *S. aureus*, MRSA, *P. aeruginosa* and *E. coli.*

iii. To determine the immunomodulatory potential of the ethyl acetate leaf extract of *O. basilicum* in Swiss albino mice?

iv. To determine the *in vivo* safety of the ethyl acetate leaf extract of *O. basilicum* in normal Swiss albino mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bacteriology of Nosocomial Pathogens

A nosocomial pathogen is that which occurs and causes disease in a patient in a healthcare facility in whom the infection was absent or in incubation at the time of hospital admission. These infections include occupational infections acquired by the staff of the health facility or those that appear after the patient has been discharged (WHO, 2002). Frequent nosocomial infections occur in surgical wounds, lower respiratory and the urinary tracts. Others are septicemia and vascular catheter infections (WHO, 2002; Mamuye, 2016).

Increase in the use of antimicrobial drugs and advances in life-saving medical practices that expose individuals to invasive procedures are associated with rising cases of nosocomial infections. Almost every pathogen has the ability to cause an infection in hospitalized individuals yet a limited number of both gram negative and gram positive bacteria are responsible for a wide range of infections (WHO, 2002; Bereket *et al.,* 2012). Among the bacteria causing nosocomial infections, *S. aureus*, *P. aeruginosa, E. coli* and Enterococci are leading with both intrinsic and extrinsic factors predisposing individuals to these pathogens (Bereket *et al.,* 2012). Other bacterial pathogens include *Klebsiella*

spp., Kluyvera spp., Proteus spp., Acinetobacter spp., Serratia marsescens among other species (Victor *et al.,* 2013).

The ability of nosocomial transmissions for a number of viruses such as hepatitis B and C, respiratory syncytial virus, enteroviruses, rotavirus, cytomegalovirus, Human immunodeficiency virus, Influenza, Ebola and herpes simplex virus exist. A number of fungi such as *Candida albicans*, *Cryptococcus neoformans, Aspergillus* spp, *Cryptosporidium* and parasites are opportunistic pathogens capable of causing infections during long periods of antibiotic treatment and severe immune-suppression (WHO, 2002; WHO 2011). The problems associated with nosocomial infections coupled with the rise in antimicrobial resistance are very relevant and their importance should not be belittled.

2.1.1 *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus*

Staphylococcus aureus is a gram positive bacterium. It is the most common cause of skin and soft tissue infections. It is transmitted primarily by direct contact including skin-skin contact with infected or colonized individuals or through contact with contaminated items and surfaces. Twenty five percent of healthy human beings are asymptomatic nasal carriers of this microbe (Belkum *et al.,* 2009; Bouvet *et al.,* 2017). It is associated with non-invasive and invasive diseases like impetigo, cellulitis, folliculitis, dermatitis, bacteremia, endocarditis, pneumonia, sepsis and toxic shock syndrome (Shittu *et al.,* 2011).

Staphylococcus aureus has developed resistance to different classes of antibiotic agents and this resistance has complicated treatment (Victor *et al.,* 2013). Of particular

importance is methicillin-resistant *Staphylococcus aureus* which has become a serious etiological agent for different infections and is one of the most common nosocomial pathogen worldwide (Nobandegani *et al.,* 2016; Belbase *et al.,* 2017).

Methicillin-susceptible *S. aureus* (MSSA) became MRSA via the acquisition and insertion of a deoxyribonucleic acid (DNA) fragment called staphylococcal chromosome cassette mec (SCCmec) into their genomes. This cassette contains mecA, the methicillin-resistance determinant. SCCmec variants have been documented. They differ in the composition of their recombinase genes and mec gene complex (Shittu *et al.,* 2011). Methicillin resistant *S. aureus* strains were once conserved to healthcare environments but since the mid-1990s, community-associated MRSA (CA-MRSA) strains that differ from health care-associated MRSA strains by infecting a dissimilar group of patients, causing clinical syndromes that are unrelated and differing in antibiotic susceptibility patterns emerged (David and Daum, 2010).

Mortality allied to severe *S. aureus* infections in the developing world far exceed those in the developed world. Studies have recognized *S. aureus* as the main causal agent of a variety of infections in sub-Saharan Africa (Mulu *et al.,* 2007; Feleke *et al.,* 2007; Olatunji *et al.,* 2007; Anguzu and Olila, 2007; Allegranzi *et al.,* 2011; WHO, 2011; Victor *et al.,* 2013).

2.1.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram negative bacilli, oxidase positive and glucose non-fermenting organism that inhabits the surfaces of aquatic environments and soil. It is an uncommon skin commensal. Its high antibiotic resistance, large genome and adaptive regulatory systems enable its survival in a variety of habitats. The resistance of this pathogen to amines and different microbicides makes its eradication from surfaces and equipment in healthcare facilities difficult (Lavoie *et al.,* 2011; Grimwood *et al.,* 2015).

Due to its nature as an opportunistic pathogen, it causes life threatening infections in individuals with damaged epithelial barriers, compromised pulmonary clearance mechanisms including individuals with non-CF bronchiectasis, cystic fibrosis, chronic obstructive pulmonary disease and immune dysfunctions. Other infections caused by *P. aeruginosa* include keratitis, UTIs, skin infections, infections of the lower and upper respiratory tract and bloodstream infections. Epithelial barriers to opportunistic infections are mostly breached iatrogenically via tracheal intubation, urinary tract catheterization and mechanical ventilation. This pathogen is one of the leading causes of ICU infections and the leading cause of ventilator associated pneumonia (Koh *et al.,* 2009; Lavoie *et al.,* 2011).

The pathogens range of virulence factors enable it to cause acute infections while its persistence in the lung is facilitated by its metabolic versatility (Koh *et al.,* 2009; Wonnenberg *et al.,* 2016). Due to the emergence of antibiotic resistant strains, there are limited therapeutic options, therefore, *P. aeruginosa* demonstrates very high morbidity

and mortality rates. The severity of its infections and the minimal antibiotic agents for treating them makes it urgent to find alternative treatment and prevention strategies (Gellatly and Hancock, 2013).

2.1.3 *Escherichia coli*

Regarded as a harmless Gram-negative bacterium, *E. coli* is part of the normal intestinal flora which has been widely used in experimental studies. Even with its nature as a normal flora, *E. coli* can become highly pathogenic due to its ability for slight genetic recombinations. Pathogenic *E. coli* is capable of causing neonatal meningitis, sepsis, bacteremia, pneumonia and travelers disease**.** Its ability to exist as a normal flora as well as a pathogen has raised the need for the development of preventive measures (Kim *et al.,* 2013).

Enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *E. coli* species are transmitted through the oral-fecal route. Enterohemorrhagic *E. coli* is capable of producing a toxin, shigatoxin that causes hemorrhagic colitis and hemolytic uremic syndromes. Enteropathogenic *E. coli* causes gastrointestinal dysfunction in elders, infants and in individuals with immune dysfunctions. These species are also capable of attaching to the intestinal epithelial cells producing attaching and effacing lesions (Yen *et al.,* 2016).

Among the most common infections in medical practice today are UTIs. It has been estimated that over 150 million people suffer from UTIs annually. According to an estimation, almost 40-50% of women experience UTIs once in their lifetime. Adult women have been reported to have recurring UTI infections. Urinary tract infections have

been reported to be a risk factor for bladder cancer and renal cell carcinoma. *Escherichia coli* is the most frequent cause of UTIs. Antibiotics greatly improved the management of these infections. However, antibiotic resistance in UTI patients represents the ballooning problem of drug resistance (Mukherjee *et al.,* 2013; Dehbanipour *et al.,* 2016,).

2.2 Risk Factors for Transmission of Nosocomial Infections

Medicolegal issues have been known to arise as a result of nosocomial infections as patients and their families sometimes blame hospital staff for the infections and demand compensation. Hospitals with nosocomial surveillance systems reduce infection rates by up to a third (Dasgupta *et al.,* 2015). Risk factors for nosocomial infections are divided into two; intrinsic and extrinsic factors. Intrinsic factors are inherent in the individual due to underlying disease conditions. Extrinsic factors are determined by the hospital staff or by practices in the whole institution (Bereket *at al.,* 2012).

2.2.1 Mechanical Ventilators

Mechanical ventilators save lives and at the same time expose associated risks to patients. Ventilator Associated Pneumonia (VAP) is a frequent nosocomial infection worldwide and continues to be a major cause of morbidity and mortality. Ventilator associated pneumonia arises from aspiration of pathogens that colonize the oropharynx. In intubated ICU patients, aspiration of these pathogens is facilitated by the depressed conscious levels that impair cough reflexes and ciliary actions. Suctioning of the trachea with contaminated medical equipment allows access of pathogens to the sterile lower respiratory tract (Joseph *et al.,* 2012; Abd-Elmonsef *et al.,* 2017).

Incidences of VAP range from 6-52%. This is an infection that occurs more than 48 hours after endotracheal intubation and mechanical ventilation has been initiated (Davis, 2006). In mechanically ventilated patients, its occurrence rate is 8-38% with a mortality rate of 20-50% (Chi *et al.* 2012; Ning *et al.* 2013).

2.2.2 The Intensive Care Unit

A multicentric study reported at least one case of ICU acquired infection in every 18.9% of patients with incidences ranging from 2.3% to 49.2%. From a point prevalence study of 1265 ICUs from 76 countries, 51% of the patients had nosocomial infections with infection rates varying among different countries. From the point prevalence study, the lungs had the highest infection rates at 64%, abdominal infections (19%) and blood stream infections at 15% (Alberti *et al.,* 2002; Vincent *et al.,* 2009).

2.2.3 Age

Novosad *et al.* (2016) in their septic study identified age as a risk factor in nosocomial infections. They reported the median age for septic adult patients to be 69 years of whom 52% were male and the rest female. On average, these individuals had stayed in hospitals for nine days. The length of stay in the hospital remains the most important determinant factor for nosocomial infections. Due to their ages, majority of the patients (97%) had comorbidities. The most common infection that led to sepsis was pneumonia (35%) followed by UTIs (25%), gastrointestinal and skin/soft tissue infections both at 11%. *Staphylococcus* spp. was the most abundant pathogen isolated from blood samples followed by *E. coli* and *Streptococcus* spp (Bereket *et al.,* 2012; Novosad *et al.,* 2016).

Comorbidities were also reported among infants. The most common health care factor reported was receipt of intravenous antibiotics in the days preceding infection. Respiratory infections followed by gastrointestinal infections were the most common (Navosad *et al.,* 2016).

2.2.4 Antibiotic Misuse and Biofilm Formation

Studies by Kumar *et al.* (2012); Venier *et al.* (2014); Wang *et al.* (2016) and Sonmezer *et al.* (2016) reported previous use of antibiotics either targeted or not targeted to the pathogen as a risk factor for infection. Loureiro *et al.* (2002) showed that prior antibiotic use was associated with bloodstream infections. The potential of bacterial pathogens to form biofilms has become quite problematic in the efforts to eradicate them. Biofilms are highly resistant to drugs. Biofilms of *P. aeruginosa* have been associated with nosocomial outbreaks with multiple antimicrobial resistance cases reported (Jefferies *et al.,* 2012).

2.2.5 Other factors

As documented by Dasgupta *et al.* (2015), studies have reported various features of primary health impairment like immunosuppression, chronic lung disease, and malnutrition as independent risk factors for infections. The use of invasive devices like tracheostomy and urinary catheterization have also been identified as important risk factors. Vincent *et al.* (2009), reported that emergency rooms, referral from the hospital floor, post emergency surgery or trauma admission, medical admission; presence of

obstructive pulmonary diseases, HIV, cancer and renal replacement therapy were independently associated with a high risk of infection.

2.3 Epidemiology of Nosocomial Infections

There is no agreement about the rate of occurrence of nosocomial infections worldwide. Burdens of these infections in different countries represent systemic failures in provision of health services (WHO, 2011). Reviews by Llata *et al.* (2009), Bereket *et al.* (2012) and Magill *et al.* (2014) reported a national prevalence of 5% as the estimate of the scope of nosocomial infections with over 90,000 deaths per year in the United States (US). Surveys have shown that *E. coli* incidences have decreased from 23 to 16% though the other pathogens continue to persist. Of the over 2 million nosocomial infections in the US, 50-60% are caused by drug resistant pathogens; 10.5% in Canada. In the west, Germany had the lowest prevalence of nosocomial infections at 3.5% while Turkey had the highest prevalence at 13.5%. Majority of the national surveys reported in the western and Asian countries had their national prevalence at between 3.5% and 13.5% with a mean of 7.1% (WHO, 2011).

In Europe and the US, UTI was the most common infection with an average of 27% and 36% respectively. The second most common nosocomial infection in Europe was lower respiratory infections at 24% followed by blood stream infections and soft tissue infections at 11%. In the US, soft tissue infections were the second most common cause of infections at 20% followed by pneumonia and bloodstream infections at 11% (Llata *et al.,* 2009; WHO, 2011).

Reviews by WHO (2011), and Nejad *et al*. (2011) on nosocomial infections brought to the fore the need for more studies on this topic in developing countries, especially African countries. The magnitude of nosocomial infections in the developing nations is underestimated because the diagnosis of nosocomial pathogens is complex while disease surveillance requires resources that are absent. The world health organization reported that the incidences of these infections in the developing world ranged from 5.7% to 19.1% with 10.1% as the pooled prevalence. In equal measure, Nejad *et al.* (2011) reported a prevalence of 5% to 15% in hospitalized patients. They documented that as many as 50% of patients in the intensive care units were infected. Surgical site infections are the most frequent in low and middle income countries with incidences from highs of 23.6% to lows of 1.2%. Surgical site infections in various nations however vary between 1.2% and 5.2%. Soft tissue infections (29.1%) were the most common followed by urinary tract infections (23.9%), bloodstream infections (19.1%) and other infections (13.1%).

In the east African region, studies in Tanzania revealed a pooled prevalence of 14.8%. After discharge, 21% of surgical patients developed infections with close to a third of them being hospitalized due to these infections (Fehr *et al.,* 2006). Another study reports that 19.4% of surgical patients developed surgical site infections while still hospitalized with the infections being identified in some patients during post discharge follow-up (Eriksen *et al.,* 2003). In Uganda, the pooled prevalence for surgical site infections was 10% with close to 9.4% of women who delivered through caesarian section being infected (Hodges and Agaba, 1997). In an Ethiopian study, the pooled prevalence of

surgical site infections among patients who had undergone abdominal surgery was reported to be 38.7% based on bacteriological criteria and 21% based on clinical criteria (Kotisso and Aseffa, 1998).

In Kenya, the pooled incidences of surgical site infections were 19%. Thirty-three percent of women who had been in labor for more than 12 hours developed surgical site infections compared to 15% for those whose labor lasted for fewer hours (Koigi-Kamau *et al.,* 2005). In a study to determine *S. aureus* carriage in Thika level 5 hospital, 8.9% of inpatient screens were reported positive for MRSA or methicillin sensitive *S. aureus* (Aiken *et al.,* 2014). Omuse *et al.* (2014) reported low levels (3.7%) of MRSA in two private hospitals in Nairobi. In their study to determine pediatric surgical site infections (SSI) in Bethany kids Kijabe Hospital, Kenya, Wood *et al.* (2012) found SSIs in 6.8% of pediatric patients. Superficial and deep infections (69% and 29% respectively) of the back and head were the most common.

2.4 The Immunobiology of Bacterial Pathogens

Stimulation of the innate and adaptive immune systems against pathogens is the hallmark of immune responses. Innate responses are activated by signal transduction pathways that detect pathogen associated molecular patterns resulting in neutrophil and macrophage activation. Neutrophils are centrally important in pathogen clearance. Adaptive immunity kicks on later during the course of infection and depends on antigen presentation by the antigen-presenting cells. Upon antigen presentation, B and T cells produce antibodies that target specific antigens. T cells and antibodies directly act against the bacteria while at

the same time aid in amplifying the activities of innate responses (Karauzum and Datta, 2016).

Understanding how plant extracts contribute to immune responses against pathogens may help identify new therapeutic strategies and could be important if harnessed for vaccine development.

2.4.1 The Innate Immune Responses

Innate immune responses were considered non-specific pro-inflammatory responses such as macrophage phagocytosis, complement activation or formation of neutrophil abscesses (Takeuchi and Akira, 2010). Advancements have since established that innate responses have considerable specificity directed towards molecular patterns in components of the pathogens called pathogen-associated molecular patterns (PAMPs). These PAMPs are recognized by specific receptors on host cells called pattern recognition receptors (PRRs). Pattern recognition receptors include Nod-like receptors (NLRs) and TLRs (Takeuchi and Akira, 2010).

Toll like receptor 4 and TLR5 recognize lipopolysaccharides and flagellin respectively after which they initiate protective immune responses. After recognition, PRRs induce the production of pro-inflammatory mediators such as chemokines, cytokines and antimicrobial peptides that initiate early immune responses (Takeuchi and Akira, 2010; Lavoie *et al.,* 2011). Several cytokines and cells are involved in modulation of the innate immune responses.

2.4.1.1 Tumor Necrosis Factor-alpha

As reviewed by Lavoie *et al.* (2011), TNF-α is produced by bone-marrow derived cells during acute infections. Signals mediated by TNF-α aid in bacterial clearance through its pro-inflammatory effects as well as its ability to induce the expression of anti-inflammatory molecules that include IL-10 and Muc1. Muc1 promotes the resolution of airway inflammation by promoting neutrophil apoptosis and suppressing TLR signaling (Cho *et al.,* 2011). Krishna and Miller, (2012) review that *S. aureus* has protein A that binds immunoglobulins in an incorrect formation thereby aiding in immune evasion by inhibiting antibody mediated phagocytosis. However, TNF receptor 1 also binds to protein A, an interaction that is of significance for host defense against *S. aureus* pneumonia.

2.4.1.2 Interleukin 1

Interleukin-1β is produced by different mechanisms during the course of bacterial infection. This may be through TLR2 or through nucleotide oligomerization domain proteins activation (Miller *et al.,* 2006; Hruz *et al.,* 2009). Interleukin-1α and IL-1β are capable of activating interleukin 1 receptor IL-1R/MyD88 signaling pathway that leads to secretion of chemokines, cytokines and adhesion molecules that promote neutrophil recruitment and abscess formation (Krishna and Miller, 2012).

Pre-made stores of immunologically active IL-1α in keratinocytes are released in cases of non-specific injury or infection. Interleukin-1β is an inducible cytokine secreted by a variety of cell types, including macrophages, keratinocytes and dendritic cells (Kupper

and Fuhlbrigge, 2004). Secretion of active IL-1β entails proteolytic processing of pro-IL-1β by caspase-1 that is dependent on formation of intracellular protein complexes known as the inflammasome (Zhou *et al.,* 2011). Interleukin-1β deficient mice have defective *S. aureus* clearance and neutrophil recruitment whereas mice deficient in IL-1α have similar phenotype to wild type mice. During intradermal *S. aureus* skin infections such as skin abscesses and cellulitis, IL-1β is the major interleukin 1 receptor (IL- 1R) ligand promoting neutrophil recruitment while during superficial *S. aureus* infections such as infected cuts, impetigo and abrasions IL-1α contributes to host defenses and neutrophil recruitment (Krishna and Miller, 2012).

2.4.1.3 Interleukin-10

Interleukin-10 is an anti-inflammatory cytokine that tends to peak late after infection. This is when chemokine and pro-inflammatory levels are diminishing. This cytokine down-regulates IL-1, TNF-a and members of the CXC and CC keratinocyte-derived chemokine families. The roles of IL-10 in infection are complex. Excess IL-10 attenuates pro-inflammatory responses while deficiency of IL-10 increases pro-inflammatory responses to infecting pathogens and endotoxins (Spight *et al.,* 2005; Muenzer *et al.,* 2010).

Staphylococcal super antigens cause massive T cell activations resulting in toxic shock syndrome and death. Despite strong adaptive responses induced by these toxins, infections by *Staphylococci* capable of producing super antigens are very common. This is partly because staphylococcal strains possess strategies that downregulate T cell

responses to these toxins. Interleukin-2 responses to staphylococcal super antigens are inhibited by the presence of *Staphylococcus*. This down regulatory effect is the result of molecules embedded in the peptidoglycan that bind to TLR 2 and induce apoptosis of antigen presenting cells and IL-10 production via the activation of PI3K-Akt cell signaling (Chau *et al.,* 2009; Peres *et al.,* 2015). Interleukin-10 decreases the expression of Human Leukocyte Antigen - antigen D Related (HLA-DR) and reduces CD86 expression on dendritic cells (Wang *et al.,* 2012).

2.4.1.4 Interleukin 17

Interleukin-17 cytokines are important in protection against infection by their ability to chemo-attract neutrophils to the infected sites by releasing the chemoattractant CXCL8 and induction of a variety of antimicrobial peptides. These cytokines are produced by different cells of the innate and adaptive immune system (Bayes *et al.,* 2016). Interleukin-17 has been shown to activate neutrophilic inflammation by producing granulopoietic factors that promote the production and maturation of neutrophils and chemokines that induce neutrophil chemotaxis (Krishna and Miller, 2012; Jaillon *et al.,* 2013). In addition, different moieties of IL-17 induce the production of different antimicrobial peptides by keratinocytes. The sustained loss of Th17 cells during HIV progression increases the risk of *S. aureus* skin colonization (Krishna and Miller, 2012).

Neutrophil recruitment mediated by IL-17 begins with the activation of IL-1α/β, TLR2 and IL-23 production that later stimulate Natural Killer cells, Neutrophils, mast cells or T cells to produce IL-17A and IL-17F. Interleukin-17A and IL-17F activates the

Interleukin 17 receptors that then produce pro-inflammatory chemokines, cytokines and adhesion molecules that mediate neutrophil recruitment (Krishna and Miller, 2012; Rigby and Deleo, 2012).

2.4.1.5 Polymorphonuclear Neutrophils

Neutrophils are the first-responder phagocytic cells recruited from circulation to sites of infection. The role played by neutrophils in infection clearance is both primary and unambiguous (Segal, 2005; Lavoie *et al.,* 2011). Neutrophil recruitment and abscess formation is required for pathogen clearance and is the most effective immune response (Molne *et al.,* 2000). Neutrophil abscess formation is mediated by a vigorous neutrophil recruitment to infection sites, survival of the neutrophils for long periods within the skin abscesses, the ability of the neutrophils to kill the pathogens and homing of c-kit+ progenitor cells to the abscess where mature cells are then produced (Kim *et al.,* 2011; Lavoie *et al.,* 2011).

Neutrophil serine proteases that include cathepsin G, neutrophil elastace and proteinase 3 are however capable of contributing to tissue inflammation and destruction by targeting host cells as well as bacterial proteins. These proteases are modulated by serine protease inhibitors such as human ova-serpin proteinase inhibitor PI9 that inhibit their activity. (Dahlen *et al.,* 1999). Rigby and DeLeo, (2012) review that neutrophils roll along the post capillary venule walls, interact with endothelial cells, survey the connective tissues, skeletal muscles, mucosal membranes and lymphatic organs for signs of tissue

inflammation, damage or pathogenic invasion. They survey by searching for the presence of host and pathogen derived chemo-attractants or chemotactic signals.

Neutrophil recruitment involves pathogen recognition by PRRs and secretion of pro-inflammatory cytokines such as TNF-alpha, IL-1β, IL-1α and IL-6. These cytokines and PRRs induce the upregulation of adhesion molecules like E-selectin, P-selectin and intercellular adhesion molecule 1 on the endothelium and lymphocyte function-associated antigen 1 and L-selectin on neutrophils that promote neutrophil rolling, diapedesis and adhesion that facilitates the neutrophils in entering the infected tissue (Fournier and Philpott, 2005; Ley *et al.,* 2007). Pro-inflammatory cytokines and PRRs also induce the production of neutrophil attracting chemokines that promote neutrophil chemo-attraction by activating CXCR2 on neutrophils (Ley *et al.,* 2007). Encounter of pathogens by neutrophils result in neutrophils expressing Fc and complement receptors thereby inducing antibody and complement mediated phagocytosis to surround opsonized bacteria into phagosomes (Segal, 2005).

Antimicrobial peptides are polypeptides that are 50 amino acids or less in length and possess antimicrobial activities at normal physiologic conditions. Neutrophils express antimicrobial peptides such as human neutrophil peptides (HNP) 1 – 4 with HNP-4 being expressed in very low amounts. These peptides constitute almost 50% of all peptides in the neutrophil granules. Human neutrophil peptide-2 exhibits the highest levels of antimicrobial activities (Ericksen *et al.,* 2005; Lehrer, 2007).

Neutrophils employ oxygen-dependent strategies to destroy invading pathogens. Production of microbicide reactive oxygen species and degranulation (fusion of cytoplasmic granules) with phagosomes accompanies phagocytosis. Degranulation provides the phagosome lumen with proteases and antimicrobial peptides which combine with the reactive oxygen species to form a non-conducive environment for the survival of the ingested pathogen (Rigby and DeLeo, 2011). Extracellular neutrophil traps are a fibrillary network composed of activated neutrophils and nuclear components such as DNA and histones. They have also been postulated to contain bound histones, chromatin cytosolic granules and azurophilic granule proteins that have the ability to bind and kill different pathogens (Rigby and DeLeo, 2011; Jaillon *et al.,* 2013).

Individuals with defective neutrophil functions are more susceptible to infections (Gonzalez-Barca, 2001; Heyworth *et al.,* 2003). Super-infection and increased colonization due to *S. aureus* has also been reported in patients with the inflammatory disease atopic dermatitis. T helper 2 cytokines such as interleukin 4 (IL-4), interleukin 13 (IL-13), interleukin 10 (IL-10) and decreased antimicrobial peptides during atopic dermatitis have been attributed to super-infection (Cho *et al.,* 2001; Ong *et al.,* 2002).

2.4.2 Adaptive Immune Responses

The adaptive immune responses are divided into cell-mediated and antibody mediated immune responses, which are facilitated by T cells and B cells, respectively (Girardi, 2007). B cell mediated responses involve production of antibodies against specific antigens. These antibodies opsonize the bacteria and facilitate antibody mediated and

complement mediated bacterial ingestion by phagocytes such as neutrophils and macrophages (Holtfreter *et al.,* 2010; Koboyashi and Deleo, 2011). The significance of B cell responses in defense against *S. aureus* is shown by the presence of *S. aureus* protein A that binds immunoglobulins in an incorrect orientation, thereby enabling the pathogen to evade antibody detection and phagocytosis (Forster, 2005; Rooijakkers *et al.,* 2005).

Pre-existing antibodies have been reported to be immunological correlates of protection in patients. Adhikari *et al.* (2012) reported that low antibody titers correspond with high risks for sepsis development following pathogen challenge. Elevated serum antibody levels against *S. aureus* alpha-hemolysin have also been correlated with protection against subsequent infection. Invasive infections have been known to produce more durable antibody responses compared to cutaneous infections (Fritz *et al.,* 2013). *Staphylococcus aureus* carriers have high titers of antibodies explaining their enhanced recovery due to an *S. aureus* challenge despite having higher risks of infection (Wertheim *et al.,* 2005).

Thymic derived T cells express T cell receptors on their surfaces that recognize antigen derived peptides on antigen presenting cells. T cells are capable of being detected after an *S. aureus* infection in humans (Zielinski *et al.,* 2012; Kolata *et al.,* 2015). Various T cell subsets have different functions. The cellular arm of the adaptive immune system comprises of the cluster of differentiation 4 (CD4+) and cluster of differentiation (CD8+) T cells. CD8+ T cells target intracellular pathogens and kill them via cytolysis of the infected cell. Depending on which activation of the CD4+ T cell receptor occurs, they are

polarized towards different effector functions with some of the polarized cells remaining in the body as memory T cells (Murphy *et al.,* 2015). Different effector subunits of CD4+ T cells have different roles.

T helper cell subsets Th1, Th2, and Th17 have been associated with the pathogenesis of *S. aureus* skin infections. T helper1 cells are produced when CD4+ T cells are primed in the presence of interleukin-12 via STAT4 signaling. T helper1 cells are capable of producing a milieu of cytokines including IL-2 and TNF-α but their signature cytokine is interferon gamma (IFN-γ) that promotes cell mediated immune responses. This cytokine activates macrophages and neutrophils to kill intracellular pathogens (O'shea and Paul, 2010). As reviewed by Karauzum and Datta (2016), Th1 cells have either non-contributory, protective or detrimental effects in case of an infection. This depends on factors including the infective titre of the pathogen, the route of infection, antigenic targets, immune balance and level of induction.

T helper 2 are produced when CD4+ T cells are primed in the presence of IL-4 via STAT6 signaling. T helper 2 produces the transcription factor GATA-3 that induces Th2 cytokines including IL-4, IL-13 and IL-5. This CD4+ T cell subset plays important roles against extracellular pathogens driving different aspects of humoral and cellular immunity to promote pathogen clearance and tissue repair (Allen and Sutherland, 2014). Their dysregulation leads to atopic and allergic diseases (Geginat *et al.,* 2013; Raphael *et al.,* 2015).

T helper 17 cells are CD4+ T cell subsets that express Rorγt and secrete an array of inflammatory cytokines such as IL-17A, IL-17F and IL-22 (Liang *et al.,* 2006; Chung *et al.,* 2006; Ivanov *et al.,* 2006). These cytokines act on epithelial cells and aid in enhancing antimicrobial properties, barrier function and neutrophil recruitment (Ouyang *et al.,* 2008). They play a protective role against extracellular fungal and bacterial infections especially in mucosal sites (Ouyang *et al.,* 2008).

2.5 Antibiotics and Antibiotic Classes

Antibiotics are very vital in managing infections. They are used in existing infections and as prophylaxis in some medical conditions and surgical procedures (Becker, 2013). Antibiotics are classified with respect to their antibacteral action and molecular structure. Antibacterial actions involve alteration of specific metabolic pathways or interruption of the synthesis of structural components of pathogens (Becker, 2013).

The beta lactam derivatives, which have a beta lactam ring that inhibit pathogenic cell wall synthesis. These drugs include the penicillins, carbapenems and the cephalosporins. Penicillins include penicillin, oxacillin, amoxicillin, dicloxacillin and ampicillin. The Cephalosporins include cefadroxil, cephalexin and cefazolin (Becker, 2013).

Macrolides express their antimicrobial action by inhibiting protein synthesis. The active compounds in these drugs bind to the 50S ribosomal unit. They include erythromycin, azithromycin and clarithromycin (Becker, 2013). Tetracyclines such as doxycycline inhibits protein synthesis by binding to the 30S ribosomal unit. Clindamycin inhibits

protein synthesis by binding to the 50S subunit of the ribosomes but unlike the macrolides, it is bactericidal (Becker, 2013).

Aminoglycosides such as gentamycin, streptomycin and tobramycin are bactericidal and inhibit protein synthesis while vancomycin inhibits cell wall synthesis. Fluoroquinolones such as ciprofloxacin, levofloxacin and gemifloxacin are the most recent advances in the field of antimicrobial therapy. They are broad-spectrum drugs known to inhibit DNA gyrase, an enzyme involved in replication, transcription, and repair of bacterial DNA (Becker, 2013).

2.5.1 Antibiotic Toxicity and Adverse Effects

Antibiotics are linked with many toxic as well as side effects. These are tolerated because treatment benefits outweigh the negatives. The side effects are well understood by clinicians who may be unable to understand other adverse events that include the overgrowth of resistant microorganisms. Pathogenic overgrowths are capable of precipitating secondary infections that can be more difficult to treat. In addition, molecular changes occurring in pathogens exposed to antibiotics are capable of enhancing their virulence. The responsibility of prescribing the right antibiotics rests with the clinicians but ignorance of potential environmental effects and an absence of microbiological understanding has led to poor prescriptions. In routine clinical care, the toxic implications of antibiotic consumption are majorly unrecognized (Dancer *et al.,* 2004).

However, some toxic effects of antibiotics have been reported. Morgun *et al.* (2015), reported that alterations in the gut due to antibiotics was because of three factors; gut microbial depletion, effects of the remaining resistant microorganisms and direct effects of the drugs to host tissues. Gut microbial depletion had the effect of downregulating different aspects of the immune system while the two other factors inhibited the amounts of active mitochondria as well as mitochondrial gene expression. Penicillins have been reported to cause stomach upsets, allergy, diarrhea, nausea, pruritus and maculopapular rashes (Becker, 2013). Macrolides have been known to inhibit cytochrome P450 enzymes that are responsible for drug interactions. When using tetracyclines, skin sensitivity to sunlight is increased leading to general erythema and intense sunburn (Ray *et al.,* 2012).

Aminoglycosides and vancomycin have been highly associated with nephrotoxicity and ototoxicity. Nephrotoxicity can be managed by discontinuing the drug in question while ototoxicity maybe permanent as sensory neurons do not regenerate. Vancomycin has been associated with histamine release that causes urticarial or erythematous reactions, tachycardia, flushing and hypotension (Becker, 2013).

2.5.2 Antibiotic Resistance Mechanisms

Antibiotics have been and continue to be the mainstay in infectious disease control. Antibiotic resistance has however developed due to a combination of factors including the transfer of genes from outside sources, antibiotic selection pressures and chromosomal mutations (Chambers and DeLeo, 2009; Tadesse *et al.,* 2012). Resistance has been documented against every class of antibiotic that has been produced, be it potent

or not. Emergence of resistance has varied in time from as low as 1 year (penicillin) to more than 10 years (vancomycin) (Tadesse *et al.,* 2012; Mamuye, 2016).

The susceptibility patterns of different pathogens vary in different geographical locations and regions. Given susceptibility patterns change through time, up to date knowledge is required to trace changes that occur with time. This is important for optimal therapy (Leegaard *et al.,* 2000; de Francesco *et al.,* 2007).

2.5.2.1 Penicillin and Methicillin Resistance

Resistance occured in waves, the first wave of resistance was to penicillin (Jevons and Parker, 1964). Methicillin was introduced as an alternative to penicillin in the 1960's and this created a second wave of resistance (Barber, 1961; Jansen *et al.,* 2006). Resistance to penicillins is due to the enzyme penicillinase, produced by pathogens, that hydrolyzes the beta lactam ring required by penicillin for its antibiotic activity (Jevons and Parker, 1964). The *mecA* gene encodes penicillin binding protein 2a while the *fem* gene confers resistance to methicillin, penicillinase-resistant penicillins and the cephalosporins (Barber, 1961; Jansen *et al.,* 2006).

Lately, MRSA isolates from Denmark, Ireland and the United Kingdom have shown a divergent *mecA* homologue called *mecC* (Garcia-Alvarez *et al,* 2011; Shore *et al,* 2011; Ito *et al,* 2012). *mecC* encodes for a penicillin-binding protein with a higher affinity for oxacillin compared to cefotoxin and a different temperature sensitivity compared to the protein encoded by *mecA* (Kim *et al,* 2012). A potential public health problem is embedded in *mecC*-positive MRSA. This is because the extended nucleotide divergence

degree between *mecA* and *mecC* means the *mecC*-positive MRSA isolates are negative when using current diagnostic procedures such as latex agglutination tests and polymerase chain reaction (PCR) assays that detect PBP2a and *mecA* respectively (Stegger *et al,* 2012).

2.5.2.2 Extended Spectrum beta-Lactamases

Extended-spectrum β-lactamases (ESBLs) are plasmid mediated, complex, diverse and rapidly evolving enzymes with the ability to hydrolize penicillins, monobactams and broad-spectrum cephalosporins. Extended-spectrum β-lactamase gene bearing plasmids also carry genes coding for resistance to other antibiotic classes such as quinolones and aminoglycosides making the selection of suitable antibacterial agents complicated. These lactams are majorly produced by *P. aeruginosa, E. coli* and *Klebsiella* spp among other spp. (Bradford, 2001; Zhao and Hu, 2010; Ruppe *et al.,* 2015).

β-lactamases spread in Gram-negatives with the aid of integrons which are able to capture and mobilize antibiotic resistance genes (Zhao and Hu, 2010). Extended-spectrum β-lactamases producing pathogens are rising in epic proportions thereby posing a clinical challenge around the world. Some countries have recorded a near 50% prevalence of ESBL producing pathogens (Bradford, 2001; Ali *et al.,* 2004; Sharma *et al.,* 2013). Extended-spectrum β-lactamases producing pathogens cause infections that range from uncomplicated UTIs to sepsis. Fluoroquinolones are used in treating UTIs though resistance is catching up with them. In severe cases, carbapenems are the drugs of

choice though resistance has been reported in a number of countries. Treatment of these infections is challenging (Ruppe *et al.,* 2015).

2.5.2.3 Carbapenemases

These are β-lactamases that hydrolize imipenem or meropenem extensively. Carbapenemases that occur naturally are encoded in the chromosomes while the acquired ones are plasmid and integrin mediated. Resistance to broad-spectrum cephalosporins by *P. aeruginosa* brought forward the use of more powerful drugs such as the carbapenems. Reports of carbapenem resistance have been documented and they have been linked to changes in outer membrane proteins (porins), acquired beta lactamase production and efflux pumps. Class A carbapenemases occur sporadically in *P. aeruginosa* while class D which have a weak hydrolyzing activity against carbapenems are rarely detected in *P. aeruginosa. Escherichia coli* has also been reported to produce carbapenemase (Tadesse *et al.,* 2002; Zhao and Hu, 2010; Dias *et al.,* 2017).

2.5.2.4 Vancomycin Resistance

Vancomycin, a glycopeptide adopted for strains not responding to β-lactams was first used in the 1950s but resistance was first reported in the 1990s with the description of vancomycin intermediate *Staphylococcus aureus* (VISA) and heterogenous VISA. Different studies have reported cases of treatment failure and prolonged bacteremia in individuals with low level vancomycin resistance. This low level resistance has been conferred by genetic and cell wall structural changes that have proved difficult to unravel (Howden *et al.,* 2014; Alam *et al.,* 2014). Vancomycin-resistance in *S. aureus* has been

attributed to the acquisition of the *vanA* gene through a conjugative transposon (Perichon and Courvalin 2009).

The VISA and vancomycin resistant *S. aureus* (VRSA) phenotypes can emerge through different mechanisms leading to some physiological and phenotypic changes including thickened cell walls, excess peptidoglycan production, reduced autolytic activity, loss of fitness, reduced biofilm formation and attenuated virulence. Vancomycin gets trapped in the thick cell wall and gets titrated to below lethal concentrations (Alam *et al.,* 2014).

2.6 Control and Prevention of Nosocomial Pathogens

Evolving aspects of nosocomial infections with regard to antibiotic resistance mean that there should be concerted efforts to control these infections from spreading. Changes in health care delivery encompassing variations in procedures and devices bring about the inevitability of infections occurring. The responsibility for controlling these infections is carried by all individuals and providers of health services (Llata *et al.,* 2009; Bereket *et al.,* 2012).

The WHO launched the first global patient safety challenge in 2005 dubbed ‘Clean Care is Safer Care’ aimed at creating a global commitment and momentum towards reducing nosocomial infections. The objectives included raising awareness of the importance of hospital acquired infections as a major component of patient safety, developing tools and guidance documents for every country to tackle the problem. Some countries such as Mali have already adopted these measures (Nejad *et al.,* 2011). The review by Nejad *et al.* (2011) also highlights other measures driven by WHO to help in the control of

infections: improving core components for infection control and prevention programs, improving the strategies for blood transfusion and injection safety, improving standards for disinfection and sterilization, safe medical waste management as well as occupational health and safety measures.

The African Partnerships for Patient Safety was launched with a political commitment aimed at improving patient care. The key areas of the plan work through improving and maintaining hospital partnerships between Europe and Africa with an emphasis on exchanging skills and knowledge among health care professionals (African Partnerships for Patient Safety, 2011).

National governments should describe full spectrums of disease burdens within their borders to help policy makers in prioritizing resources and inform stakeholders on efforts to combat nosocomial infections. Most importantly, the formulated policies should be put into effective practice (Llata *et al.,* 2009; Aiken *et al.,* 2014; Magill *et al.,* 2014). The outcomes of national control and prevention strategies will rely on the participation of healthcare providers who are knowledgeable on nosocomials. It is therefore necessary that valid training on prevention and accountability be done to ensure the validity of these efforts (Llata *et al.,* 2009).

Staff related procedures such as hand hygiene should be emphasized to reduce the spread of these pathogens. Gowns, masks and gloves are important in controlling the spread of infections but their use is haphazard (Al-Hamad and Maxwell, 2010). The minimum required resources and facilities including microbiological facilities should be available

for infection control and case reporting. Research should also be done on potential patient and family involvement in infection control and reporting as well as in adaptation of valid surveillance protocols founded on the realities in the developing nations (WHO, 2011). Antimicrobial susceptibility patterns must and should be documented to serve as points of reference when medicating against nosocomial infections. This will reduce the cases of drug resistance in subsequent infections and provide objective measures when tracking resistance trends and intervention evaluations thereby informing overall recognition, prevention and treatment efforts (Omuse *et al.,* 2014; Novosad *et al.,* 2016).

Addressing disparities in nutrition, education and hygiene is essential in the control of surgical site infections. Other risks for nosocomial infections regionally should be defined so that academic and clinical resources are directed towards addressing them (Wood *et al.,* 2012).

2.7 *Ocimum basilicum* and Herbal Management of Infectious Diseases

Nature gives us both problems and solutions. It is up to us as its inhabitants to find the solutions, problems will find us. With infectious diseases, nature has been a relied upon source of medicinal agents for ages. Over 50% of the conventional medicines have been derived from natural sources. In many developing countries, plants are still being used for therapeutic effects against many diseases. The WHO estimates that 80% of the earth's population rely on traditional medicine with the use of plant extracts or their active components being predominant (Singh and Watal, 2010; Devendran and Balasubramanian, 2011; Nabavi *et al.,* 2015).

As antibiotic resistance continues to run scientists in circles and roundabouts, interest in natural products as potential sources of antibacterial agents has increased. Many infectious diseases have been treated with herbal formulations (Al-Bayati, 2009). Antibacterial compounds can be derived from stems, barks, flowers, leaves or fruits of various plants (Gordon, 2001). Limitations on antifungal and antibacterial agents brought about by drug resistance has given us food for thought. Even though pharmacological advancements have produced new drugs, their subsequent use has led to antimicrobial resistance (Singh and Watal, 2010).

Secondary plant metabolites have different roles and it can be postulated that their ecological roles have a bearing on their use as medicinal options. Among secondary metabolites, terpenoids, polyphenols, alkaloids, polypeptides, lectins and polyacetylenes are antimicrobial agents. These metabolites are recognized as being generally safe as food products with negligible side effects (Nabavi *et al.,* 2015). The metabolites with cytotoxic effects against plant pathogens could have therapeutic effects against human pathogens, if not toxic to the humans themselves. Plant metabolites have the ability to be effective against different pathogens without providing the ability of these pathogens to develop adaptive or resistance responses (Singh and Watal, 2010; Nabavi *et al.,* 2015).

Ocimum basilicum is a herb belonging to the kingdom plantae, order lamiales, family lamiaceae, genus *Ocimum* and species *O. basilicum*. This plant is native to Asia, Africa and Pacific Island (Ismail *et al.,* 2004). It has formed part of medicinal plants used widely as a food additive and as an antimicrobial. It has antinociceptive and antioxidant effects

among others (Araujo *et al.,* 2015). Traditionally, its leaves are used as spices in cuisines while its oils have been used to flavor sausages and meat. Basil oil has traditionally been used in antimicrobial products (Sappakul *et al.,* 2003).

Ocimum basilicum extracts have antimicrobial activities on fungi such as *Fusarium* spp. (Hanif *et al.,* 2010; Kocic-Tanackov *et al.,* 2011) while Runyoro *et al.,* 2010 reported that oils of *Ocimum* spp did not have antimicrobial effects against different species of fungi, Gram positive and gram negative bacteria (Shirazi *et al.,* 2013; Vlase *et al.,* 2014; Joshi, 2014; Al Abbassy *et al.,* 2015).

Studies on *O. basilicum* showed that it contains oil constituents such as methyl chavicol, geranial, linalool, *epi*-α-cadinol, neral, caryphyllene oxide and *trans*-α-bergamotene (Sajjadi, 2006; Hanif *et al.,* 2010; Bayala *et al.,* 2014). Hanif *et al.* (2010) documented that *Ocimum* oils did not have any antibacterial activity on selected bacteria while Sakkas and Papadopoulou (2017) in their review reported different studies showing the antibacterial activities of *Ocimum* oils. The antimicrobial activities, chemical composition and their antioxidant activities depend on the type of soil and seasonal variations (Hussain *et al.,* 2008)

Figure 2.1: Sweet basil (*Ocimum basilicum*) (Source: Authors).

2.7.1 Phytochemicals and Antimicrobial Mechanisms

Plant secondary metabolites including alkaloids, tannins and flavonoids among others have been shown to have antimicrobial effects. They inhibit antimicrobial growth via a variety of ways. In their review, Lee and Lee, (2015) report that the antimicrobial actions of various phytochemicals vary and may include cell wall damage, cytoplasmic membrane damage, formation of reactive oxygen species, DNA fragmentation, phosphatidylserine externalization, metacaspase activation, mitochondrial membrane

depolarization, nuclear condensation, modulation of transcription factors, redox signaling and redox-sensitive transcription factors (Omojate *et al.,* 2014).

Phytochemicals exert their antimicrobial action by resembling endogenous hormones, ligands, metabolites, neurotransmitters or signal transduction molecules therefore expressing beneficial effects to humans (Omojate *et al.,* 2014). Exploitation of these factors can help in the development of better antimicrobial approaches.

2.7.1.1 Flavonoids

These are phenolic substances with more than one benzene ring. They possess antiviral activities. They include kaempferol, quercetin, and quercitrin. Flavonoids also encompass flavones, flavans, dihydroflavons, flavonols, catechin, anthocyanidins, calchones, proanthocyanidins, and leucoanthocyanidins (Omojate *et al.,* 2014). These phytochemicals form complexes with cell walls and bind to bacterial adhesins inhibiting them from binding to epithelial surfaces (Tiwari *et al.,* 2011). The flavonoid Isoquercitrin interferes with cell membrane integrity. Flavones such as amentoflavone increase the concentration of reactive oxygen species inside cells resulting in cellular damage, they form complexes with extracellular soluble proteins and form complexes with cell walls. They also result in mitochondrial dysfunction and caspase activation (Lee and Lee, 2015).

2.7.1.2 Alkaloids

The major composition of plant secondary metabolites are the alkaloids. They are comprised of nitrogen bases with a variety of radicals containing oxygen replacing a

number of the hydrogen atoms in a peptide ring (Omojate *et al.,* 2014). These substances integrate into the DNA and interfere with the biochemical integrity of the cell walls (Tiwari *et al.,* 2011). Berberine has been shown to increase the reactive oxygen species inside microorganisms resulting in cellular damage (Lee and Lee, 2015).

2.7.1.3 Phenols, Polyphenols and Tannins

Polyphenols or phenols are chemical components occurring ubiquitously as color pigments for fruits. They are phenylalanine derivatives. Tannins are polymeric phenolic substances formed by flavan derivative condensations transported to woody tissues (Omojate *et al.,* 2014). These substances bind to adhesins, deprive microorganisms off substrates, inhibit enzymatic activities, form complexes with cell walls and interfere with cell membrane permeability. Some polyphenols act by inhibiting virulence factors of some pathogenic microorganisms (Tiwari *et al.,* 2011; Daglia, 2012).

Phenols such as eugenols have been documented to inhibit antimicrobial growth by damaging the integrity of the cell wall, interfering with the permeability of the cell membrane. Tannins are also capable of interfering with bacterial metabolic enzymes. They bind bacterial dihydrofolate reductase enzymes, inhibit the supercoiling activity of *E. coli* gyrase enzyme by binding to the adenosine triphosphate (ATP) binding sites (Omojate *et al.,* 2014). It then binds bacterial DNA inducing topoisomerase IV mediated DNA cleavage and bacterial stasis. Pyrogallol and catechol have toxic activities on microbes. Physiological activities in humans including the stimulation of phagocytic cells

and host-mediated tumor activities are linked to tannins (Omojate *et al.,* 2014; Lee and Lee, 2015).

2.7.1.4 Quinones

Aromatic rings composed of two ketone substitutions. They are highly reactive. Quinones inactivate microbial enzymes, bind the microbial adhesins and form cell wall complexes disturbing the integrity of the cell walls. They form irreversible complexes with amino acids in proteins therefore resulting to losses in cellular functions (Tiwari *et al.,* 2011; Omojate *et al.,* 2014).

2.7.1.5 Saponins

Saponins are 'soap' like substances and produce an aglycone on hydrolysis called sapogenin. Saponins alter cell wall permeability and combine with bacterial membranes to alter cell morphology leading to lysis. Alpha tomatine has been reported to have inhibitory functions against fungi and also leads to nuclear condensation (Omojate *et al.,* 2014; Lee and Lee, 2015).

2.7.1.6 Glycosides

Glycosides are products of sugar condensation with varieties of thiol or organic hydroxyl or thiol compounds with the hemiacetal moiety of the carbohydrate playing an ignoble role in the reaction. Examples include cardiac glycosides, anthracene glycosides, chalcone glycoside, amarogentin, rographolide, gentiopicrin, polygalin and ailanthone (Omojate *et al.,* 2014). Glycosides such as glycans damage the integrity of microbial cell walls (Lee and Lee, 2015).

2.7.1.7 Terpenoids and Essential Oils

Terpenes are the most chemically widespread and diverse group of natural products. Terpenoids such as sterols insert themselves between phospholipids of the microbial cell membranes interfering with the permeability of the cell membranes. This interferes with the osmotic conditions inside the cell and destabilizes ion transport. The terpenoid carvacrol inhibits bacterial biofilm formation (Omojate *et al.,* 2014; Lee and Lee, 2015).

2.7.1.8 Lectins and Polypeptides

Plants express one or more lectins each with a unique ability to select and bind to different carbohydrate structures. Lectins differ in carbohydrate specificity, molecular structures and biological functions. They include mannans that damage the integrity of microbial cell walls (Lee and Lee, 2015).

2.8 Immunomodulatory Effects of Plant Extracts

The immune system is important in protection against diseases. Immunomodulation refers to the alteration of the immune system by interfering with its functions, if it enhances immune reaction, it is immunostimulative, if it suppresses the immune system functions, it is referred to as immunosuppressive. To regulate normal immune functions, both immunosuppression and immunostimulation should be considered. Immunostimulating and immunosuppressing compounds have their standing, the search for compounds that exert these activities is becoming the norm around the world (Sahu *et al.,* 2013).

Infectious diseases that compromise the body's immune system have brought forward the need to find alternative ways of boosting the immune system (Nfambi *et al.,* 2015). Plant products as immune stimulating agents is a topic under research. Plant secondary metabolites such as lectins, flavonoids, polysaccharides, peptides and tannins have immune stimulating potential. Immune modulators are capable of stimulating the adaptive and the innate defense systems thereby helping the body in its defense against pathogens (Makare *et al.,* 2001).

Delayed type hypersensitivities (DTH) are vital parts of graft rejection, immunity to intracellular pathogens and tumorigenesis. Delayed type hypersensitivities require antigen recognition by T lymphocytes leading to cytokine proliferation and release. These cytokines are responsible for vascular vasodilation, permeability, macrophage chemoattraction and activation, increased phagocytic activities and improved lytic concentrations (Sahu *et al.,* 2013). Sahu *et al.* (2013) have reported that the herbal formulation of *Habenaria intermedia* increase DTH responses and improves phagocytic activities. Various studies using different plants have shown corresponding results (Sharififar *et al.,* 2009; Tripathi *et al.,* 2010; Sheyab *et al.,* 2012; Nfambi *et al.,* 2015).

Some phytochemicals such as flavonoids induce the apoptosis of quiescent and preferentially activated T cells. In experimental asthmatic conditions, the flavonoid luteolin decreased IL-4 and IL-5 and increased IFN-γ in broncho-alveolar fluids while epigenin lowers IgE, RANTES, IL-4 and IL-13. Quercetin reduced eosinophil infiltration but not neutrophil infiltration and had no effect on IL-5. Polyphenols inhibit the

expression of proinflammatory cytokines (TNF, IL-1β, and IL-6) while quercetin increases IL-10 production at low concentrations (Gonzalez *et al.,* 2011).

Mice receiving polyphenol rich oral treatment from date palm tree increased immune cells such as Th1, macrophages, natural killer cells and dendritic cells. The same results were shown in aged rats treated with *Cassia auriculata* polyphenols. These polyphenols increased splenic B and T cells (Cuevas *et al.,* 2013). Epigallocatechin-3-gallate decreased Th1 and Th17 populations while baicalin reduced 1L-17 cytokines. Polyphenols repress the secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-alpha. When murine cells were treated with *Chinese propolis*, they increased the mRNA expression levels of IL-6 and IL-1β. Treatment with *Lonicera japónica* (*Caprifoliaceae*) and *Kalanchoe gracilis* crude extracts reduced the expression of TNF-α, IL-6, IL-1-β (Lai *et al.,* 2011; Park *et al.,* 2012; Wang *et al.,* 2013). In their review, Spelman *et al.* (2006) corroborate these findings while, using different plants, some of the findings are contradicted by Mani *et al.,* (2015).

2.9 Mineral elements and Heavy Metals

Human beings need complex organic compounds taken as caloric requirements to meet their needs for muscular activities. Fats, carbohydrates and proteins are a major part of the diet with minerals and vitamins forming a smaller part of the diet. Studies have shown that optimal intakes of mineral elements such as K, Na, Mg, Mn, Ca, Cu, Zn can reduce risk factors associated with cardiovascular diseases. Significance of mineral elements in

preventing diseases is being studied across the world (Indrayan *et al.,* 2005; Rahmatollah and Mahbobeh, 2010; Soetan *et al.,* 2010).

Due to pollution, exposure to toxic metals is a growing problem worldwide. The misuse of hazardous metals in industries and agriculture and their poor disposition has created risks for animals, plants and humans. Heavy metals are very reactive, accumulative and exceptionally toxic when their concentrations are above the threshold. The World Health Organization recommends that herbal formulations be checked for contaminants such as heavy metals, microorganisms and pesticides. The excess and deficiency of essential micro-elements such as iron, zinc and copper have harmful effects on humans. Toxic metals such as cadmium, chromium, lead, mercury, arsenic and nickel have deleterious effects on human health (Pinto *et al.,* 2011). The use of plants to detect the accumulation, deposition and distribution of heavy metals has been done for long. Recently, the use of leaves as bio-monitors of heavy metal pollution has been increasing (Hussain and Khan, 2010; Pinto *et al.,* 2011).

Phosphorous is a constituent of adenosine triphosphate, nucleic acids, tooth and bone formation. Phosphorous is significant for normal heart contractions, maintaining blood sugar levels, cell growth and repair, kidney functions and bone growth. It is an important element in maintaining the acid-base balance in the body (Nile and Khobragade, 2009; Soetan *et al.,* 2010). Iron is needed in red blood cell functions, in hemoglobin, the oxygen carrying pigment. It is a component of the cytochromes functioning in cellular respiration. It helps in the formation of ligaments and tendons in the body as well as

controlling some chemicals of the brain (Nile and Khobragade, (2009); Soetan *et al.,* 2010; Rahmatollah and Mahbobeh, 2010).

Magnesium, Cu, Se, Zn, Fe and Mn are enzyme co-factors that are important in biochemical pathways including ATP generation, and for physiologic processes such maintenance of cardiovascular and neuromuscular tone. Sodium, K and Ca are of significance in maintaining osmotic balance (Soetan *et al.,* 2010; Rahmatollah and Mahbobeh, 2010). Potassium is an intracellular cation involved in electrical excitation and membrane potential of muscle and nerve cells (Rahmatollah and Mahbobeh, 2010).

Magnesium is important in muscle and bone formation, helps prevent depression, high blood pressure and is important for enzyme activities. Deficiency of Mg leads to poor nerve transmissions, poor impulses, nervousness and irritability. Calcium is significant in bone and teeth formation and is an important part of blood and extracellular fluid. Blood coagulation, cell permeability regulation, milk clotting and cardiac muscle functions need Ca. Its deficiency leads to irritability, osteoporosis, back pain, indigestion, rickets, premenstrual tension and cramps (Nile and Khobragade, 2009).

Selenium is an essential trace mineral element of significance to human health. It has enzymatic and structural roles functioning as an antioxidant and as a catalyst for active thyroid hormone production. This mineral is needed for immune functions, inhibition of the progression of HIV to AIDS, sperm motility and reduces the risks of miscarriage. Adverse mood states have been linked to deficiency in selenium (Rayman, 2000).

Copper is an essential element for many enzymes such as lysyl oxidase, cytochrome oxidase and ceruloplasmin which is a blood enzyme that oxidizes Fe. Its sources include fertilizers, pesticides, sewage sludge and industries. Copper is also capable of causing neurological disorders such as irritability, depression, muscular pains and nervousness and other effects such as skin and hair discoloration (Hussain and Khan, 2010; Shah *et al.,* 2013).

Zinc accumulates from burning fossil fuels, sewage, agrochemicals and non-ferrous smelters. Zinc is important in protein and nucleic acid metabolism, tendons and ligaments. About 85% of the Zn in blood combines with protein for transport. High concentrations lead to high density lipoprotein levels and a decrease in immune function. Zinc deficiency results to coma, diarrhea, abnormalities in fetal development, hypogensia, pneumonia and diabetic hyposomia (Hussain and Khan, 2010; Pinto *et al.,* 2011).

Manganese is an important element whose uptake is regulated metabolically. Its deficiency leads to reproductive and skeletal abnormalities. High concentrations are hazardous to the brain and lungs. Manganese in soils come from fertilizers, ferrous smelters and sewage sludge (Hussain and Khan, 2010; Shah *et al.,* 2013).

Nickel is found combined with Oxygen or Sulphur in the environment. It’s important in lipid content regulation and red blood cell formation. High concentrations lead to body weight loss, poor vision, allergic dermatitis, carcinogenesis, lung, heart and liver failures (Hussain and Khan, 2010; Shah *et al.,* 2013).

Lead is toxic to plants, animals, humans and microorganisms. This element is sourced from mines, sewage slugs, fuel combustion and farm yard manures. Lead causes multiple endocrine effects like those connected with renal dysfunctions and male fertility. It also leads to pale skin, nausea, anemia, abdominal pain, birth defects and miscarriages, vomiting and wrist joint paralysis. Effects on the nervous system leads to delirium, intellectual disabilities, coma and death. (Hussain and Khan, 2010; Pinto *et al.,* 2011; Sharma *et al.,* 2015).

Chromium is a vital element with its sources being tannery, sewage sludge, steel industries, chromium alloys and plating in motor vehicles. Chromium stabilizes blood sugar levels, helps in absorbing energy from blood and increases muscle fat (Nile and Khobragade, 2009). In excess, Cr is associated with stomach ulcers, skin rashes, respiratory problems, kidney and liver damage, weakened immune systems, altered genetic material and death (Hussain and Khan, 2011).

Cadmium is a naturally occurring metal found as an impurity in Zn and Pb deposits. It is used in lasers, T.V screens, cosmetics, batteries, paint pigments and galvanizing steel. Cadmium is highly toxic and is sourced from fertilizers, zinc and lead smelters, sewage sludge and fuel combustion (Hussain and Khan, 2010). Cadmium induces tissue damage by creating oxidative stress, upregulation or inhibition of transport pathways, causing epigenetic changes in DNA, interfering with physiologic actions of Mg or Zn, heme

synthesis inhibition, mitochondrial function impairment, glutathione depletion and structurally distorting proteins (Bernhoft, 2013).

Mercury is a malleable liquid at normal temperatures and pressure. Sources of mercury comprise of earthquakes, volcanic activity, erosion and mercury volatilization in vegetation and marine environments. In the central nervous system, Hg leads to an increase in reactive oxygen species, a decrease in the number of neurons and inhibits tubules necessary for central nervous system development. In the cardiovascular system, it leads to myocardial infarction, hypertension, atherosclerosis and coronary dysfunctions (Azevedo *et al.,* 2012).

Arsenic is an element that occurs in many minerals in combination with Sulphur, metals and in pure elemental forms. Aberrant Hedgehog pathway when activated inhibits tumor growth. This helps in inhibiting the growth of cancers of various tissues and organs. This pathway has been studied in animal models with the aim of developing therapeutic strategies against cancer. Arsenicals inhibit this pathway by targeting Gli, the primary activator of hedgehog-dependent transcription (Kim *et al.,* 2010).

2.10 Safety of Plant Extracts as Therapeutic Agents

Plants as sources of medicinal compounds are almost free from the adverse effects caused by the conventional drugs. Due to their cultural acceptability and less side effects, they are widely used for medication by almost 80% of the world's population. The standards for herbal safety have been published by WHO in order to avoid abuse and adulterations (Doughari *et al.,* 2009). For herbal formulations with polyphrnon E, resveratrol and

curmin, only minor adverse effects have been documented (Boocock *et al.,* 2007). Even with their safety, use of herbal extracts with conventional drugs should be monitored as they may lead to herb-drug interactions that are clinically relevant. These herb-drug interactions can have very adverse effects (Liu *et al.,* 2009).

Hypericum perforatum used to treat depression, interacts with HIV inhibitors, digoxin, warfarin and theophylline. Some medicinal plants on ingestion affect the isoenzymes of cytochrome P450 or phosphoglycoprotein transporter systems thereby inhibiting drug distribution and excretion. Conventional medicines and herbal medicines when used concurrently lower blood plasma concentrations of conventional drugs. This leads to low therapeutic doses circulating or toxic concentrations (Phillipson, 2007).

Phytochemicals have biphasic dose effects on mammalian cells. High concentrations are toxic while sub toxic doses induce adaptive stress responses including signal pathway activations resulting in increased gene expression to form cytoprotective proteins. Hermetic mechanisms of action have been suggested to underlie phytochemical health benefits (Mattson, 2008). Phytochemicals also act as antioxidants protecting cells against the effects of reactive oxygen species such as superoxide, singlet oxygen, hydroxyl radicals and peroxyl radicals. Reactive oxygen species are capable of leading to cellular stress and cell damage. These antioxidants are important in preventing chronic and degenerative diseases such as cardiac and cerebral ischemia, atherosclerosis, carcinogenesis, rheumatic disorders, diabetic pregnancy, ageing and DNA damage (Uddin *et al.,* 2008; Jayasri *et al.,* 2009).

Several studies have absolved plants off toxic effects. Liao *et al.* (2017) in their review discussed that many sources of Chinese traditional medicine were safe for use. Different studies done on traditional African medicine have shown that herbal formulations are safe for use (Awounfack *et al.,* 2016).

2.11 Biochemical and Hematological Markers of Toxicity

Efforts to bring new drugs into the world are greatly undermined by toxicity. As many as 92% of all new compounds developed for therapeutic purposes fail to enter the market due to adverse effects reported in clinical development. Routinely assessed toxicological parameters are blood, renal and hepatic indices. Crucial roles of the blood tissue, the hepatic and renal organs in drug transportation, detoxification and excretion evokes a plethora of drug related complications that maybe toxic (Fuchs and Hewitt, 2011).

Toxicity as a result of herbal medicines and synthetic drugs depend upon the route of administration as well as exposure. Tissue damage due to cellular destruction can have a hematological, biochemical or immunological basis. Mechanisms of pathologic lesions are unknown in the stage between toxin interactions with cellular constituents and the beginning of degenerative changes leading to cell death (Arika *et al.,* 2016).

2.11.1 Biochemical Markers

2.11.1.1 Liver Biomarkers

The roles played by the liver in the metabolism and excretion of xenobiotics increases its susceptibility to toxic and adverse effects (Dollah *et al.,* 2013). Over 27% of the drugs withdrawn from the market are as a result of hepatotoxicity while over 40% terminations

of drugs in clinical phases are due to hepatotoxicity (Chang *et al.,* 2011). Liver damage can be diagnosed by some biochemical markers such as liver enzymes including alanine transaminase (ALT), alkaline phosphatase (ALP) and Aspartate transaminase (AST). Liver function assessment can also be used to gauge liver damage by evaluating the levels of bilirubin, albumin and total proteins in blood.

Alanine transaminase is an enzyme that’s normally present in the heart and liver cells. Damage to the heart or liver increases the presence of ALT in the bloodstream. When liver cells are damaged, enzymes found in the cytosol are released into the bloodstream with their presence in plasma being an important marker of the type and extent of hepatocellular damage. Aspartate transaminase, ALP and ALT are enzymes used to regularly inform on the integrity of liver cells. With their predominance in the liver, ALT and ALP are more hepato-specific compared to AST which is found in equal ratios in the heart, kidney, liver, muscles and the brain. Elevations of ALT three times above the normal is considered a sign of hepatic toxicity. The ALT: ALP ratio is important in determining the type of liver damage produced by toxins. A ratio equal to or greater than five signifies hepatocellular damage while a ratio less than or equal to two signifies cholestatic liver damage. Cholestasis leads to impaired bile flow, jaundice and itching. High ALP levels are also a sign of hepatobiliary injury (Chang *et al.,* 2011; Singh *et al.,* 2011; Dollah *et al.,* 2013).

A rise in bilirubin levels to more than twice its normal ranges signify hepatic damage. Total bilirubin is raised when there is reduced hepatic clearance leading to cholestasis and hepatobiliary injury. Accumulation of triglyceride leads to steatosis that can be manifested by a large droplet or small droplet fatty liver. Albumin and total proteins are produced by the liver, therefore, their levels are a marker of liver damage (Singh *et al.,* 2011).

2.11.1.2 Kidney Biomarkers

The kidney consists of nephrons that are capable of filtering about 150-180 liters of plasma in a single day then processing the filtrate to regulate acid-base balance and electrolytes while removing waste products. Hormones responsible for hematologic, cardiovascular and skeletal muscle homeostasis are also produced by the kidney. Nephrotic toxicity is manifested in drug development and during clinical care. Drug toxicity contributes to about 19-25% of all cases of acute nephrotic injury in critical patients. Two serum biomarkers, creatinine (CRE) and urea have been used as markers for nephrotoxicity (Bonventre *et al.,* 2010).

2.11.1.2.1 Creatinine

Creatinine is a product of the breakdown of creatinine phosphate in muscles and is produced depending on muscle mass. It is used as a measure of nephrotic functions. Diagnoses of kidney failure are done when creatinine levels in serum exceed the upper limits of normal intervals. Reduction in creatinine excretion occurs in uremia and chronic renal failure (Gowda *et al.,* 2010; Dollah *et al.,* 2013).

2.11.1.2.2 Urea

Urea is the end product of amino acid and protein catabolism. It's distributed by the liver and circulated in the intracellular and extracellular fluids. Filtration of urea out of the blood is done in the kidneys by the glomeruli with part of it being reabsorbed as water. Urea concentration in serum is used to estimate renal function. Blood urea is linked to renal disease or failure, kidney stones that block the urinary tract, dehydration, congestive heart failure, bleeding and shock in the digestive tract (Gowda *et al.,* 2010; Dollah *et al.,* 2013).

2.11.1.3 Hematological Markers

Hematological parameters such as red blood cell indices, white blood cell indices can be assessed and used as diagnostic markers of adverse effects caused by foreign agents in the bloodstream of animals.

2.11.1.3.1 Red Blood Cells and Related Indices

Red blood cells (RBCs) are packed with hemoglobin, the oxygen carrier. A low number of RBCs is associated with anemia. Toxins are capable of stimulating the lipid peroxidase system resulting in the secretion of lipid peroxides that hemolyse RBC's (Kolanjiappan *et al.,* 2002; Junqueira *et al.,* 2006). Hemoglobin (Hb) is the intracellular protein found in RBC's. It binds oxygen in the lungs for transport to tissues and also binds carbon dioxide from the tissues for transport to the lungs for exhalation (Wintrobe and Greer, 2009). Blood cell destruction leads to low Hb levels (Junqueira *et al.,* 2006), while high Hb levels can be attributed to hemolysis of red blood cells due to inflammation or antibody attack (Ugwu *et al.,* 2013).

Hematocrit (HCT) is the RBC volume percentage in whole blood and is used as a signal for anemia. It is a determinant of blood viscosity with high blood viscous levels contributing to insulin resistance (Wintrobe and Greer, 2009). Mean corpuscular volume (MCV) denotes the average size of the RBCs. Low MCV denotes microcytic, normal MCV denotes normocytic while high MCV denotes macrocytic RBCs. Low MCV is related to Fe deficiency, thalassemia and microcytic anemia while high MCnVs are related to chronic alcoholism, folate and vitamin B12 deficiency (Aslinia *et al.,* 2006). Mean corpuscular hemoglobin (MCH) denotes the average amount of Hb inside an RBC. Mean corpuscular hemoglobin concentration (MCHC) denotes the average concentration of hemoglobin inside a RBC. It is used in diagnosing Fe deficiency (Namrata, 2013).

2.11.1.3.2 White Blood Cells and Related Indices

White blood cells are vital in immune functions. They arise from pluripotent hematopoietic stem cells in the bone marrow (myeloid). Granulocytes (GR) such as neutrophils and monocytes are phagocytic (Arika *et al.,* 2016). Monocytes are also essential in the recognition and interactions of immune cells and antigens. Lymphocytes (LY) aid phagocytes in defense against foreign invasion. These cells develop from the bone marrow and thymus (Hoffbrand *et al.,* 2006).

2.11.1.3.3 Platelets (PLT)

These are dynamic blood particles important in coagulation, hemostasis and blood clotting. Along with leucocytes, they search for injured sites and become activated thereby changing their shape, increase their surface area and secrete active biomolecules stored in their alpha and dense granules molecules (Lopez *et al.,* 2015). Platelets also

play a role in the inflammatory process, wound healing, microbial host defense, angiogenesis and remodeling (Golebiewska and Poole, 2015).

Mean platelet volume (MPV) denotes the amount of platelets in the bloodstream. High MPV indicates enlarged platelet diameter, which is a marker of platelet production rate and activation. Platelet distribution width (PDW) indicates volume variability in platelet size and is raised in the existence of platelet anisocytosis (Budak *et al.,* 2016). Platelet distribution width measures platelet size variability, platelet activation changes thereby reflecting heterogeneities in platelet morphology (Vagdatli *et al.,* 2010; Budak *et al.,* 2016). Plateletcrit (PCT) is the percentage volume of platelets in the blood. A simultaneous reduction in platelet counts and PCT indicates an excessive consumption of platelets (Zhang *et al.,* 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of *O. basilicum*

The plant used in this research, *Ocimum basilicum*, was ethnobotanically identified with information about its use among the Mbeere community, in Kenya being gleaned from a local traditional herbalist. As the plant is not an endangered species, no permission was needed prior to its collection. Fresh leaves were collected from natural habitats in Makunguru Village, Nthawa Location, Siakago Division, Mbeere North Sub-County, Embu County, Kenya on longitude 29° and between latitudes 0°35'38"S and 37^{0}38'12"E (Figure 3.1). Botanical identification of the plant was done by a taxonomist and a voucher specimen deposited at the National Museum Herbarium, Nairobi for future reference. The voucher number WAM-XVI was assigned.

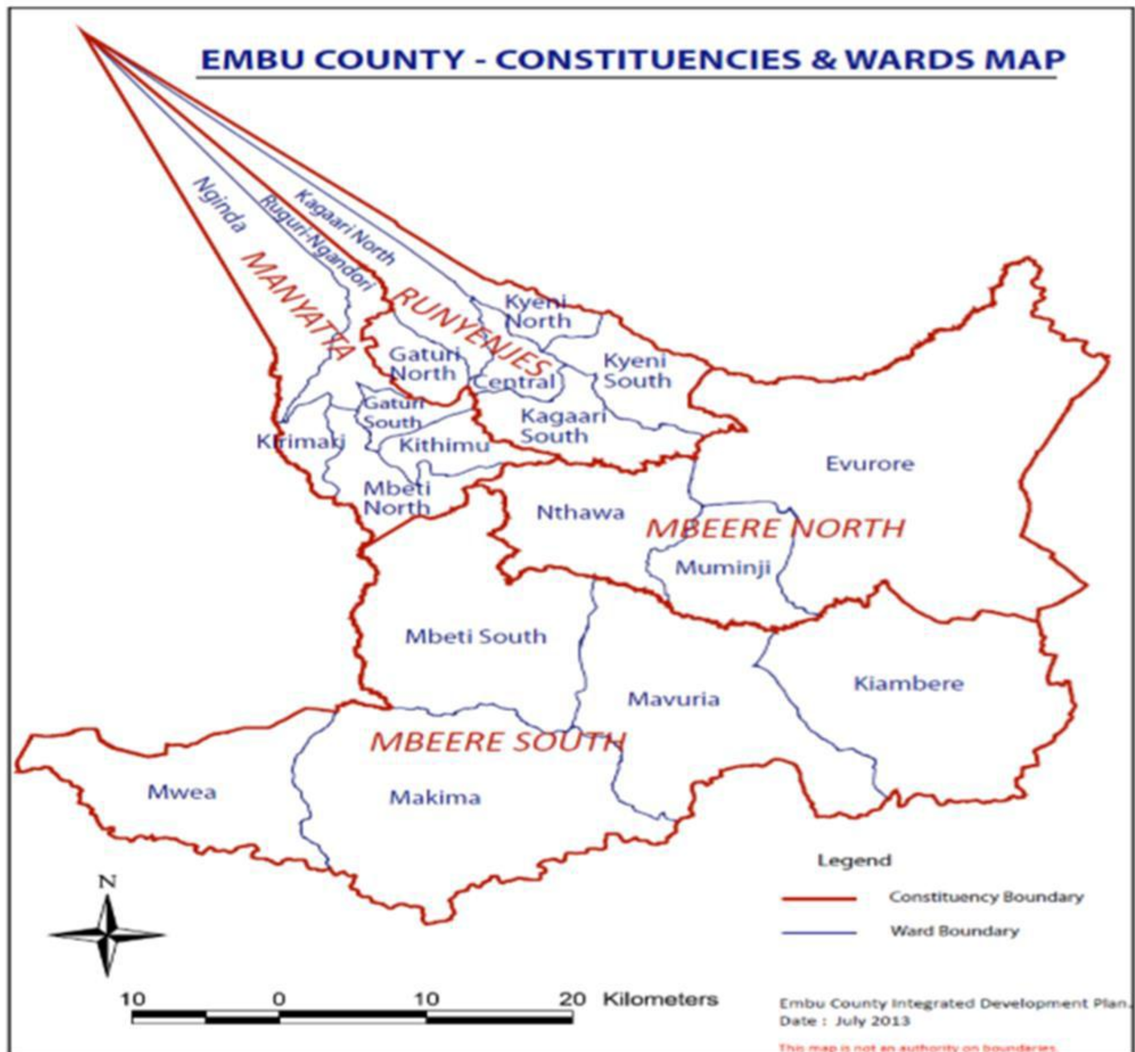


Figure 3.1: Nthawa location, Embu County from where the plant was collected, retrived from Arika *et al.* 2015.

3.2 Processing and Extraction

Processing and extraction was done in the Pharmacy, Complementary and Alternative Medicine Departmental laboratory, Kenyatta University. The freshly collected leaves were washed under running tap water and air dried at room temperatures away from

direct sunlight for a period of four weeks. The dried leaves were milled into fine powder by an electric mill.

Five hundred grams of the powdered *O. basilicum* leaves were soaked in 1.5 liters of ethyl acetate (LOBA Chemie, Mumbai, India) in a volumetric flask for 72 hrs. The solution was sieved using a muslin cloth and filtered using a Rocker 400 vacuum pump (Rocker Scientific, New Taipei, Taiwan) into a conical flask. To obtain the concentrate from the crude mass extract, vacuum drying of the semisolid mass was done using a rotary evaporator (Rocker Scientific, New Taipei, Taiwan). Evaporation of ethyl acetate (boiling point – 77.7^{o}C) from the crude extract was done at a temperature of 79°C for 2 hours to obtain a solid residue (concentrate or yield). The percentage yield was calculated on a dry weight basis. The extract was refrigerated at 4 ^{o}C untill use. This procedures were adopted from previously published (Sui *et al.,* 2016). Pilot studies were done to ascertain the antibacterial activity of the extract and to optimize the research protocols.

3.3 Spectrophotometric Methods

3.3.1 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was done according to the procedure by Nyamai *et al.* (2015). One gram of the extract was weighed into a 1.5 ml Eppendorf tube and dissolved in 1 ml of ethyl acetate. The mixture was vortexed for 30 seconds and sonicated for five minutes using a Branson 2510E-DTE sonicator. The sample was spun in a centrifuge for 5 minutes at 1300 rpm. The supernatant was transferred into 2 ml auto

sampler vials and analyzed using a Gas Chromatograph Mass Spectrometry (7683 Agilent Technologies, Inc., Beijing, China).

3.3.2 Quantitative Mineral Element and Heavy Metal Analysis

For the quantitative analysis of mineral elements and heavy metals, the procedure as described by Shah *et al.* (2013) was used. Concentration of the mineral elements and heavy metals was obtained after concentration, extraction and digestion with Nitric acid followed by analysis using the atomic absorption spectrophotometer. Hydrogen peroxide at 30% and 65% nitric acid were used for digestion. A multi-element standard solution was used for the digestion purposes.

For sample digestion, a solution of nitric acid and hydrogen peroxide was prepared in a 2:1 ratio. One gram of the dried, powdered plant material was dissolved in the solution. The sample was then heated on a hot plate fixed at 130^0C to increase its solubility untill the volume was reduced to 3 ml. Cooling was done and the solution filtered into a volumetric flask with the aid of a Whatman filter paper and diluted up to the mark. Mineral element analysis was finally done using an Analytik Jena ContrAA 700 atomic absorption spectrophotometer (Jena, Germany). This analysis was done in triplicate.

The concentration of Phosphorous was determined using the Vanadomolybdo phosphoric Acid Colorimetric method (Stuffins, 1967). The principle for this method is that in dilute orthophosphate solutions, under acidic conditions, ammonium molybdate reacts to form molybdophosphoric acid, which is a heteropoly acid. Yellow vanadomolybdophosphoric

acid is formed in the presence of Vanadium. The concentration of phosphate is equal to the intensity of the yellow colour. One ml of the sample was dispensed into a clean cuvette and 0.25 ml of vanadate-molybdate reagent (Labchem Cat #LC266002) added. The sample was then mixed by pipetting several times and left for 10 minutes until the colour developed. The calorimeter was calibrated against distilled water, after which the cuvettes with the samples were placed into it, measured and the readings done. Readings were done three times after every calibration.

3.4 *In vitro* Antibacterial Assays

3.4.1 Extract Dosage Preparation for Antibacterial Assays

Four extract concentrations used in *in vitro* antibacterial assays were prepared using 4% Dimethyl sulphoxide (DMSO). These were 1 g/ml, 0.75 g/ml, 0.50 g/ml and 0.25 g/ml which corresponded to 100%, 75%, 50% and 25% concentrations, respectively. These concentrations were put in sterile bijou bottles and refrigerated at 4 ^{0}C during use.

3.4.2 Bacterial Strains

Four quality control strains with American Type Culture Collection numbers (ATCC) were used in this study; two Gram negative bacteria (*E. coli* ATCC 29211 and *P. aeruginosa* ATCC 27853) and two Gram positive bacteria (*S. aureus* ATCC 25923 and MRSA ATCC 43300). They were obtained from archived cultures at the Pharmacy, Complementary and Alternative Medicine departmental laboratories, Kenyatta University. Clinical isolates of *E. coli, S. aureus* and *P. aeruginosa* were gifted to me by

Mr. Jonathan Mateba of the department of Biochemistry, Microbiology and Biotechnology, Kenyatta University.

The bacteria were sub-cultured on nutrient agar medium (Himedia Laboratories, India), and incubated at 37 ^{o}C for 18 hours. The fresh growing bacterial colonies were transferred using a sterile wire loop into capped sterile glass tubes containing nutrient broth medium (Himedia Laboratories, India). The inoculated broth was incubated at 37 ^{o}C for 24 hours. The bacterial suspensions were then maintained at 4 ^{o}C as stock cultures.

3.4.3 Inoculum Preparation and Antibacterial Assays

Antibacterial assays were performed according to the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines. These assays were performed in the Medical Laboratory Sciences departmental laboratories at Kenyatta University. Microbial cultures from the stock suspensions were inoculated onto the surface of Mueller Hinton Agar [MHA] (Mast group ltd, Merseyside, UK) using a sterile swab and incubated for 18 hours to obtain fresh growing colonies.

A fresh growing bacterial colony was picked from a petri dish using a sterilized wire loop, inoculated into 4 ml of sterile peptone water (Himedia Laboratories, India) and incubated at 37°C for 4 hours. The bacterial suspensions were then adjusted using peptone water in order to obtain turbidity comparable to 0.5 McFarland’s standard, which corresponds to about $1\text{-}2\times10^{8}$ colony forming units/ml (CFU/ml). These suspensions were used within fifteen minutes of preparation.

Determination of antibacterial susceptibility patterns was done by the agar disc diffusion method as per Njeru *et al.* (2016) with slight modifications. Gentamycin (40 mcg/ml) and Neomycin (200 mcg/ml) (Sigma Aldrich, United Kingdom) were used as positive controls. Paper discs were prepared from a Whatman number one filter paper using a paper punch, placed in bijou bottles and sterilized. Aliquots of 0.1 mL of the bacterial suspensions were aseptically pipetted and inoculated by spread plate method on the Mueller hinton agar (Mast Group Ltd, UK). The paper discs were impregnated with 20 µl of each concentration of the plant extract, and control antibiotics and placed on the surface of the inoculated petri dishes using sterile forceps. Dimethyl sulphoxide at 4% was used as the negative control. Each disk was pressed against the agar medium to ensure level and complete contact. The agar plates were inverted and incubated at 37 ^{o}C for 18 hours and the diameters of the zones of inhibition measured using a ruler. These tests were done in triplicates.

Letters were used to denote different antimicrobial agents as: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D - Gentamycin; E - Neomycin; F – Dimethyl Sulphoxide; G – 0.25 g/ml Extract.

3.4.3.1 Determination of Minimum Inhibitory Concentrations (MIC)

The broth microdilution method, as previously described (Njeru *et al.,* 2016) with minor modifications, was used to determine the MIC on Mueller Hinton broth medium. A bacterial colony of each test bacterium was picked using a sterilized wire loop and placed into 4 ml of Mueller Hinton broth medium in test tubes and incubated at 37 ^{o}C for 4

hours. The bacterial suspensions were then adjusted to 0.5 McFarland's standard. One hundred µl aliquots of the inoculated broth at McFarland's standard were placed into each well of the 96 well microtiter plates (Nalge Nunc international, Denmark).

One hundred microliters of the 1 g/ml concentration of the extract was pipetted and added into each of the first well of the inoculated microtiter plate and serial diluted eight times. The obtained extract dilutions were 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90624 mg/ml. One hundred microliters of Gentamycin were pipetted into the control wells and serial diluted eight times. The concentrations obtained were 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 µg/ml. One hundred microliters of neomycin were also pipetted into the control wells and serial diluted seven fold. The obtained concentrations were 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 µg/ml. Dimethyl sulphoxide was used as the negative control. An aluminum foil was placed on top of the microtiter plates to prevent dehydration during incubation. The microtiter plates were incubated at $37^{o}C$ for 18 hours. The MIC was considered as the lowest concentration of the plant extract, and controls, at which no visible growth (turbidity) was observed after incubation. This test was done in triplicates.

3.4.3.2 Determination of Minimum Bactericidal Concentration

The incubated microbial cultures from which the MIC values were obtained were used as the inocula. The MHA medium was prepared and dispensed into petri dishes. Fifty µl aliquots of the incubated microbial cultures in 96 micro well plates at the MIC level were pipetted and sero-diluted to the ninth dilution. Fifty µl aliquots of the ninth dilution were pipetted and inoculated onto the surface of the agar plates and spread using a sterile

applicator stick. Fifty μl aliquots of the bacterial suspensions in the wells immediately above and below (+1 and -1) the MIC well were also serial-diluted and inoculated onto the surface of the agar plates. The same procedure was repeated for the control drugs.

The inoculated plates were incubated at 37 ^{o}C for 18 hours. The lowest concentrations that exhibited the complete inhibition of bacterial growth were taken as the minimum bactericidal concentrations (MBC). This test was done in duplicates.

3.5 Determination of *In vivo* Immunomodulatory Activities

Determination of immune modulation was done using three therapeutic doses (100, 200 and 300 mg/kg body weight) of the plant extract.

3.5.1 Drugs

Cyclophosphamide (Zuviphos - Zuvius Lifesciences Limited, Mumbai, India, Batch no; PCYCKZ601; Expiry; April – 2019) was used as an immunosuppressant. Levamisole Hydrochloride BP 40 mg syrup (Laboratory and Allied Pharmaceuticals Limited, Nairobi, Kenya, batch no; 68552; Expiry date: 03, 2020) was used as a positive control drug. All the reagents and chemicals used were of analytical grade and checking was done to confirm they were not expired before use.

3.5.2 Antigen Preparation

Fresh rabbit blood was obtained from the animal breeding and handling facility in the department of Biochemistry, Microbiology and Biotechnology of Kenyatta University. Rabbit red blood cells (RRBCs) were subsequently washed three times in phosphate buffered pyrogen free normal saline (Appendix I). An RRBC concentration of 0.1 ml

comprising 1 x 108/mm^3 cells (10%) was attained by using phosphate buffered normal saline.

3.5.3 Experimental Animals

Swiss albino mice of either sex with an average age of 8±1 weeks and an average weight of 27±3 g, were obtained from the animal breeding and handling facility in the department of Biochemistry, Microbiology and Biotechnology of Kenyatta University. The mice were kept in standard propylene cages and provided with mice pellets and tap water *ad libitum*. Housing was done under normal conditions (22 ± 5^0C).

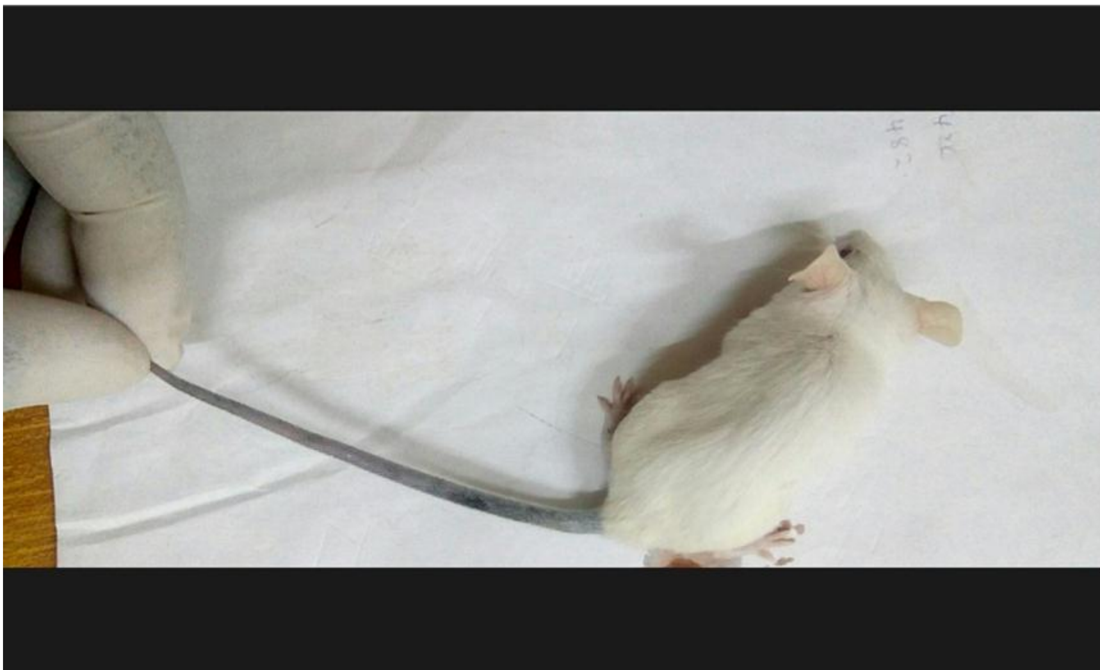


Figure 3.2: The Swiss Albino Mice (Source: Authors')

3.5.4 Experimental Designs for the Immunomodulatory Effects of the Ethyl acetate Leaf Extract of *O. basilicum*

3.5.4.1 Determination of the Effect of the Ethyl Acetate Leaf Extract of *O. basilicum* on Cyclophosphamide Induced Myelosuppression

This was determined according to the procedure described by Gaikwad *et al.* (2011). Briefly, the animals were randomly distributed into six groups of five mice each. On the 1st day, blood was obtained from the tail vein of each mice in each group and their hematological parameters assayed. Thereafter, dose administrations were performed as follows:

Group I (normal control) mice were intragastrically administered with 0.2 ml of 4% DMSO from the 2nd to the 14th day. Group II (positive control) mice were intragastrically administered with Levamisole hydrochloride at a dose of 50 mg/kgbw from the 2nd to the 14th day. Group III (negative control) mice were intragastrically administered with 0.2 ml of 4% DMSO from the 2nd to the 14th day. Group IV mice were intragastrically administered with the extract at a dose of 100 mg/kgbw from the 2nd to the 14th day. Group V mice were intragastrically administered with the extract at a dose of 200 mg/kgbw from the 2nd to the 14th day. Group VI mice were intragastrically administered with the extract at a dose of 300 mg/kgbw from the 2nd to the 14th day.

For groups II, III, IV, V, and VI, mice were also subcutaneously administered with 0.2 ml cyclophosphamide (20 mg/kgbw) from the 12th to the 14th day of the study period. Blood

was obtained from the tail vein on the 15th day and assayed for hematological parameters. This study design is summarized in table 3.1.

Table 3.1: Experimental Design for the Determination of the Effect of Ethyl Acetate Leaf Extract of *O. basilicum* on Cyclophosphamide Induced Myelosuppression

		Treatment			
Group	**Day 1**	**Day 2 – 11**	**Day 12 – 14**	**Route**	**Day 15**
I	Blood collection	DMSO	DMSO	i.g	Blood collection
II	Blood collection	Lev 50 mg/kgbw	Lev + CYP	i.g + s.c	Blood collection
III	Blood collection	DMSO	CYP	s.c	Blood collection
IV	Blood collection	Extract (100 mg/kgbw)	Extract + CYP	i.g and s.c	Blood collection
V	Blood collection	Extract (200 mg/kgbw)	Extract + CYP	i.g and s.c	Blood collection
VI	Blood collection	Extract (300 mg/kgbw)	Extract + CYP	i.g and s.c	Blood collection

DMSO – Dimethyl sulphoxide; CYP, cyclophosphamide; S.c., subcutaneous injection; Lev, levamisole hydrochloride; S.c i.g., intragastric route.

3.5.4.2 Determination of Hemagglutinin Antibody Titers and Neutrophil Adhesion Tests

This was done according to the procedures as described by Nfambi *et al.* (2015) with minor modifications. Thirty Swiss albino mice were randomly distributed into six groups of five mice each and dose administrations performed as follows;

Group I mice (normal control) were intraperitoneally administered with 0.2 ml of 4% DMSO and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were administered with 0.2 ml of 4% DMSO. On the 8th day, mice were again sensitized with RRBC's while intragastric DMSO dosing continued from the 9th to the 15th day.

Group II mice (negative control) were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were administered with 0.2 ml of 4% DMSO. On the 8th day, mice were again sensitized with the RRBCs while intragastric DMSO dosing continued from the 9th to the 15th day.

Group III mice (positive controls) were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of levamisole hydrochloride (50 mg/kgbw). On the 8th day, mice were again sensitized

with RRBCs while Levamisole hydrochloride administration continued from the 9th to the 15th day.

Group IV mice were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 100 mg/kgbw. On the 8th day, mice were again sensitized with the RRBCs while extract administration continued from the 9th to the 15th day.

Group V mice were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 200 mg/kgbw. On then 8th day, mice were again sensitized with the RRBCs while extract administration continued from the 9th to the 15th day.

Group VI mice were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 300 mg/kgbw. On the 8th day, mice were again sensitized with RRBCs while extract administration continued from the 9th to the 15th day. This design is summarized in table 3.2.

Table 3.2: Experimental Design for Determination of Hemagglutinin Antibody Titer and Neutrophil Adhesion Tests

			Treatments		
Group	**Day 1**	**Day 2-8**	**Day 8**	**Day 9-15**	**Day 15**
I	DMSO+RRBCs	DMSO	RRBCs	DMSO	Blood collection
II	CYP+RRBCs	DMSO	RRBCs	DMSO	Blood collection
III	CYP+RRBCs	50 mg/kgbw Lev	RRBCs	50 mg/kgbw Lev	Blood collection
IV	CYP+RRBCs	Extract (100 mg/kgbw)	RRBCs	100 mg/kgbw	Blood collection
V	CYP+RRBCs	Extract (200 mg/kgbw)	RRBCs	200 mg/kgbw	Blood collection
VI	CYP+RRBCs	Extract (300 mg/kgbw)	RRBCs	300 mg/kgbw	Blood collection

DMSO – Dimethyl sulphoxide; CYP, cyclophosphamide; Lev, levamisole hydrochloride; RRBC's – Rabbit Red Blood Cells.

On the 15th day, blood was collected by cardiac puncture from all the study groups. For humoral antibody tests, the procedure as described by Gaur *et al.* (2009) was adopted. Serum was separated by centrifugation at 4000 rpms for ten minutes. Serial dilutions of the serum with normal saline were then done in 96 well microtiter plates. Twenty five µL aliquots of 1% RRBCs were added to each of the dilutions. Incubation of the microtiter plates was done for 1 hour at 37°C and examined for agglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the hemagglutinnin antibody titer (HA units/µL).

For the neutrophil adhesion test, blood was analyzed for total leucocyte count (TLC) and differential leucocyte counts (DLC). The blood samples were then incubated with nylon fibers (80 mg/ml) for 15 min at 37°C. After incubation, blood was assayed for TLC and DLC again. Neutrophil index (NI) was reported as the product of TLC and % neutrophil of the blood sample. Percentage neutrophil adhesion was determined by the formula (Nfambi *et al.,* 2015):

$$\text{Neutrophil adhesion } = \frac{\text{Niu} - \text{Nit}}{\text{Niu}} \times 100$$

where, Niu - neutrophil index of untreated blood sample; Nit - neutrophil index of treated blood sample.

$$\text{Neutrophil index (NI)} = \text{TLC} \times \%\ \text{Neutrophil adhesion}$$

3.5.4.3: Determination of RRBC - Induced Delayed Type Hypersensitivity (DTH) Responses, WBC and Platelet Counts

This assay was done according to the procedure as described by Nfambi *et al.* (2015). Thirty Swiss albino mice were randomly distributed into six groups of five mice each. Group treatments were done as follows;

Group I mice (normal control) were intraperitoneally administered with 0.2 ml of 4% DMSO and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were administered with 2 ml of 4% DMSO. On the 8th day, mice were again sensitized with RRBCs while intragastric DMSO dosing continued from the 9th to the 15th day.

Group II mice (negative control) were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were administered with 0.2 ml of 4% DMSO. On the 8th day, mice were again sensitized with RRBC's while oral DMSO dosing continued from the 9th to the 15th day.

Group III mice (positive control) were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of levamisole hydrochloride at a dose of 50 mg/kgbw. On the 8th day, mice were again sensitized with RRBCs while Levamisole hydrochloride administration continued from the 9th to the 15th day.

Group IV mice were intraperitoneally administered with 0.2 ml of cyclophosphamide at 20 mg/kgbw and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 100 mg/kgbw. On the 8th day, mice were again sensitized with the RRBCs while extract administration continued from the 9th to the 15th day.

Group V mice were intraperitoneally administered with 0.2 ml of cyclophosphamide at 20 mg/kgbw and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 200 mg/kgbw. On the 8th day, mice were again sensitized with RRBCs while extract administration continued from the 9th to the 15th day.

Group VI mice were intraperitoneally administered with 0.2 ml of cyclophosphamide (20 mg/kgbw) and sensitized with 0.1 ml of 10% (1x 10^8) RRBCs on the 1st day. From the 2nd to the 8th day, mice were intragastrically administered with 0.2 ml of the extract at a dose of 300 mg/kgbw. On the 8th day, mice were again sensitized with RRBCs while extract administration continued from the 9th to the 15th day. This design is summarized in table 3.3.

Table 3.3: Experimental Design for RRBC - Induced Delayed Type Hypersensitivity Responses, WBC and Platelet Counts

			Treatments			
Group	**Day 1**	**Day 2-8**	**Day 8**	**Day 9-15**	**Day 15**	**Day 18**
I	DMSO +RRBCs	DMSO	RRBCs	DMSO	RRBCs	Blood collection
II	CYP+RRBCs	DMSO	RRBCs	DMSO	RRBCs	Blood collection
III	CYP+RRBCs	50 mg/kgbw Lev	RRBCs	50 mg/kgbw Lev	RRBCs	Blood collection
IV	CYP+RRBCs	Extract (100 mg/kgbw)	RRBCs	100 mg/kgbw	RRBCs	Blood collection
V	CYP+RRBCs	Extract (200 mg/kgbw)	RRBCs	200 mg/kgbw	RRBCs	Blood collection
VI	CYP+RRBCs	Extract (300 mg/kgbw)	RRBCs	300 mg/kgbw	RRBCs	Blood collection

DMSO – Dimethyl sulphoxide; CYP, cyclophosphamide; Lev, levamisole hydrochloride; RRBCs – Rabbit Red Blood Cells; O.B, *O. basilicum* ethyl acetate leaf extract.

On the 15th day, mice in all treatment groups were challenged with 0.2 ml of 10% RRBCs in the left hind paw through the subcutaneous route. Phosphate buffered saline at a pH of 7.4 was injected in the right hind paw as a control. Footpad edema was measured with the aid of a Vernier caliper at 0hr, 2hr, 4hr, 6hr, 8hr, 12hr, 24hr, 36hr and 48hr. Delayed type hypersensitivity reactions were expressed as a percentage increase in footpad edema over the control values.

$$\text{DTH} = \frac{\text{Diameter of Left pootpad} - \text{Diameter Right footpad (control)}}{\text{Diameter of Left footpad}} \times 100$$

On the 18th day, blood was obtained by cardiac puncture and analyzed for WBC and platelet counts.

3.5.4.4 Determination of Carbon Clearance for Phagocytic Activity

This was done according to the method described by Cheng *et al.* (2005). Mice were randomized into six treatment groups of five mice each. Group treatments were done as:

Group I mice (normal control) were administered with 4% DMSO from the 1st to the 14th day. Group II mice (negative control) were administered with cyclophosphamide (20 mg/kgbw) from the 1st to the 14th day. Group III mice (positive control) were administered with the reference drug Levamisole hydrochloride at a dose of 50 mg/kgbw from the 1st to the 14th day. Group IV mice were administered with the extract of *O. basilicum* at a dose of 100 mg/kgbw from the 1st to the 14th day.

Group V mice were administered with the extract of *O. basilicum* at a dose of 200 mg/kgbw from the 1st to the 14th day. Group VI mice were administered with the extract of *O. basilicum* at a dose of 300 mg/kgbw from the 1st to the 14th day. On the 14th day, each mouse was weighed and later injected with 0.1 ml of 1:50 diluted carbon ink through the tail vein. Twenty μl of whole blood was then sampled via the retroorbital plexus of each mouse at the 2nd and 15th minute. After blood collection, mice were euthanized and sacrificed to obtain the liver and spleen whose weights were determined. This experimental design is summarized in table 3.4.

Table 3.4: Experimental Design for Carbon Clearance for Phagocytic Activity

		Treatment
Group	**Day 1-14**	**Day 14**
I	DMSO	Carbon ink
II	CYP	Carbon ink
III	Lev	Carbon ink
IV	100 mg/kgbw Extract	Carbon ink
V	200 mg/kgbw Extract	Carbon ink
VI	300 mg/kgbw Extract	Carbon ink

DMSO: Dimethyl Sulphoxide; CYP: cyclophosphamide; Lev: levamisole hydrochloride

The blood was lysed with two milliliters of 1% disodium carbonate and the absorbance determined at 680 nm. Carbon clearance index (K) was calculated as:

$$\text{Rate of Carbon Clearance (K)} = \frac{\text{Log OD2} - \text{Log OD15}}{\text{T2} - \text{T1}}$$

The Phagocytic index was calculated as:

$$\text{Phagocytic index } = \frac{\frac{\text{K1}}{3} \times \text{Body weight of animal}}{\text{Liver weight} + \text{Spleen weight}}$$

3.6 28-Day Repeated-dose Oral Toxicity Study in Mice

In accordance with the Organization for Economic Cooperation and Development test guidelines (OECD, 2008) and the US Food and Drug Administration (FDA) Toxicological Principles for the Safety Assessment of Food Ingredients, 28-Day Repeated-dose oral toxicity study was done to determine the potential toxic effects of the ethyl acetate leaf extract of *O. basilicum* in Swiss albino male mice.

3.6.1 Experimental Mice

Twenty Swiss albino male mice weighing 25±2 g and aged 6-8 weeks were obtained from the stock at the animal breeding and handling facility in the department of Biochemistry, Microbiology and Biotechnology, Kenyatta University. The mice were placed in propylene cages for the duration of the study. Provision of mice pellets and tap water *ad libitum* during experimentation was adhered to according to the National

Guidelines for the Care and Use of Animals in Research and Education in Kenya (CNHR, 2016).

3.6.2 Experimental Design

A completely randomized controlled study design was employed in this study. Dosage concentrations were prepared according to the method published by Oghenesuvwe *et al.* (2014). Experimental protocols as described by Arika *et al.* (2015) and Kimang'a *et al.* (2016) were employed in this study, with minor modifications. Dimethy sulphoxide (4%) was used as the solvent and vehicle. The 20 Swiss albino male mice were randomly distributed into three experimental groups and one control group of five mice each. The experimental group mice were intragastrically administered with the extract doses of 300, 548 and 1000 mg/kg body weight daily for 28 days. The control group was administered with 4% DMSO daily for 28 days. The mice were observed for behavioral changes, illness and mortality during treatment. Their weights were measured once weekly during the dosing period. This experimental design is summarized in table 3.5.

Table 3.5: Experimental Design for Safety Evaluation

Group	Group Description	No. of Mice	No. of Days
I	4% DMSO	5	28
II	300 mg/kgbw Extract	5	28
III	548 mg/kgbw Extract	5	28
IV	1000 mg/kgbw Extract	5	28

DMSO – Dimethyl Sulphoxide

3.6.2.1 Blood Collection

At the end of the 28 days, mice were euthanized using chloroform and blood collected by cardiac puncture using a syringe and a hypodermic needle. About 1.5 ml of blood from each animal was dispensed in heparin anticoagulated vacutainer tubes for biochemical assays while another 1.5 ml was dispensed in ethylene diamine tetra-acetic acid (EDTA) vacutainer tubes for hematological assays.

3.6.2.2 Hematological Assays

Whole blood in EDTA tubes was used for assaying hematological parameters using the Coulter counter system (Beckman and Dickinson Coulter®, U.S) according to the manufacturer's instructions. The parameters determined include red blood cell indices such as Hemoglobin, Mean Corpuscular Hemoglobin, Mean Corpuscular Volume, Red Blood Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin Concentration and Haematocrit; White blood cell indices such as Lymphocytes, Monocytes (MO) and Granulocytes; Platelet counts and platelet indices such as Mean Platelet Volume, Plateletcrit and Platelet Distribution Width.

3.6.2.3 Biochemical Assays

The blood in heparin anticoagulated tubes was centriguged at 5000 rpms for ten minutes, plasma was pipetted and dispensed into vacutainer tubes. The Olympus 640 Chemistry Analyzer was used to determine liver and kidney functions. The liver function markers determined include Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin (ALB), Total protein (TP), total bilirubin (TBIL),

direct bilirubin (DBIL), triglycerides (TG) and total cholesterol (TC). The Renal function markers included Urea (UREA) and Creatinine (CRE).

3.6.2.4 Absolute Organ Weights and Organ Indices

Organs were excised from mice and weighed. The organs obtained include the heart, lungs, liver, brain, kidneys, spleen and testis. Organ indices were determined using the formula described by Tripathi *et al.* (2010).

$$Organ\ Index = \frac{Weight\ of\ Organ}{Body\ Weight}$$

3.7 Ethical Approval

The study was done in line with the National Guidelines for the Care and Use of Animals in Research and Education in Kenya (CNHR, 2016) and the principles for good laboratory practice (OECD, 1998). Ethical approval for use of experimental animals was sought from the Kenyatta University Ethical Committee.

3.8 Data Analysis

Both qualitative and quantitative data was collected. Quantitative data was entered into the Ms Excel sheet. The data was found to conform to the assumptions of parametric data using box plot. Descriptive statistics (mean ± standard deviation) were used to express continuous variables. To determine statistical significance, the students T-test was used for comparisons of antibacterial activities between leaf extracts and the control drugs. The essential difference in the effects of the ethyl acetate leaf extract of *O. basilicum* on different immune system and toxicity markers was calculated using one way analysis of

variance (ANOVA). A p value of ≤ 0.05 was set as the threshold for statistical significance; when significant effects occurred, Tukeys post hoc test was performed. Data analysis was done using the statistical software Minitab version 17. Data is presented in tables and graphs.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield

The percentage yield of the ethyl acetate leaf extract of *O. basilicum* used in the study was determined as:

$$Percentage\ Yield = \frac{Weight\ of\ Final\ Extract}{Weight\ of\ the\ Soaked\ Sample} \times 100$$

$$Percentage\ Yield = \frac{16.02}{500} \times 100 = 3.2\%$$

Therefore, the solvent used (ethyl acetate) was able to extract 3.2% of the plants natural products for use in this study.

4.2 Phytochemical Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

The GC-MS analysis of the ethyl acetate leaf extract of *O. basilicum* revealed the presense of 30 compounds. Based on the obtained results, 2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (Nootkatone), a sesquiterpenoid was found to be of the highest concentration (20.86 mg/g). 1,3-Dimethyl-5-isobutylcyclohexane, a fatty acid, was found to be of the lowest concentration (0.10 mg/g). The retention time, molecular formula, the chemical class and the concentration (mg/g) of the identified compounds in the *Ocimum basilicum* ethyl acetate leaf extract are presented in table 4.1 and figure 4.1.

Table 4.1: Phytochemical Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

RT (min)	Phytochemical compound	Molecular Formula	Chemical Class	Conc (mg/g)
4.9188	Oxirane, 2-methyl-2-(1-methylethyl)-	$C_6H_{12}O$	Fatty acid	0.12
6.6882	9-Thiabicyclo[3.3.1]non-6-en-2-amine, N-methyl-, endo-	$C_9H_{15}NS$	Amine	0.26
8.6367	1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl-	$C_6H_{12}N_2$	Phenol	0.47
8.9055	1,3-Dimethyl-5-isobutylcyclohexane	$C_{12}H_{24}$	Fatty acid	0.10
9.4654	3,6,6-Trimethyl-cyclohex-2-enol	$C_9H_{16}O$	Phenols	1.96
9.6894	1-Butanol, 3-methyl-, carbonate (2:1) (Diisopentyl carbonate)	$C_{11}H_{22}O_3$	Ester	0.27
11.2572	9,10-Dehydro-6-desoxy-indolinocodeine	$C_{18}H_{19}NO_2$	Alkaloids	0.14
20.1489	Tetradecanal	$C_{14}H_{28}O$	Aldehydes	3.10
22.3886	Tridecenol<2E->	$C_{13}H_{26}O$	Aldehydes	2.15
22.7021	5-Aminomethyl-5-oxo-1,3,5-diazaphosphorinane	$C_{14}H_{17}N_3O_4$	Amine	0.65
24.4939	Manool oxide	$C_{20}H_{34}O$	Diterpene	0.57
25.5018	2-Methoxyamphetamine	$C_{10}H_{15}NO$	Amphetamine	0.15
26.2185	E-11(13-Methyl)tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	Aldehyde	0.34
26.9352	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$		0.18

27.4503	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	Fatty acid	1.73
29.1749	6,3'-Dimethoxyflavone	$C_{17}H_{14}O_4$	Flavanoids	1.82
29.9364	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-	$C_8H_{13}N_7$		1.40
30.6531	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	Alkaloid	5.77
31.1235	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$	Triterpene	2.69
32.0418	Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester	$C_{18}H_{18}ClNO_3S$	Ester	1.44
34.3487	Eicosane, 10-heptyl-10-octyl-	$C_{35}H_{72}$	Essential oils	3.19
35.8269	1H-Pyrazole, 1-(3-methylbutyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-	$C_{14}H_{25}BN_2O_2$	Esters	2.92
39.724	.beta.-Amyrin	$C_{30}H_{50}O$	Triterpene	7.75
40.2391	1,2,4,8-Tetramethylbicyclo[6.3.0]undeca-2,4-diene (Isocaryophyllene)	$C_{15}H_{24}$	Sesquiterpene	6.72
40.7543	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (Nootkatone)	$C_{15}H_{22}O$	Sesquiterpene	20.86
42.1429	Zierone	$C_{15}H_{22}O$	Sesquiterpene	11.45
42.882	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	$C_{12}H_9F_2NO_3$	Alkaloid	0.93

43.0612	9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-	$C_{18}H_{18}O$	Phenol	0.60
43.3075	A'-Neogammacer-22(29)-en-3-ol, acetate, (3.beta.,21.beta.)- (Lupeol acetate)	$C_{32}H_{52}O_2$	Triterpene	16.76
46.7119	l-Alanine, N-(2-thienylacetyl)-, butyl ester	$C_9H_{14}F_3NO_3$	Phenol	0.25

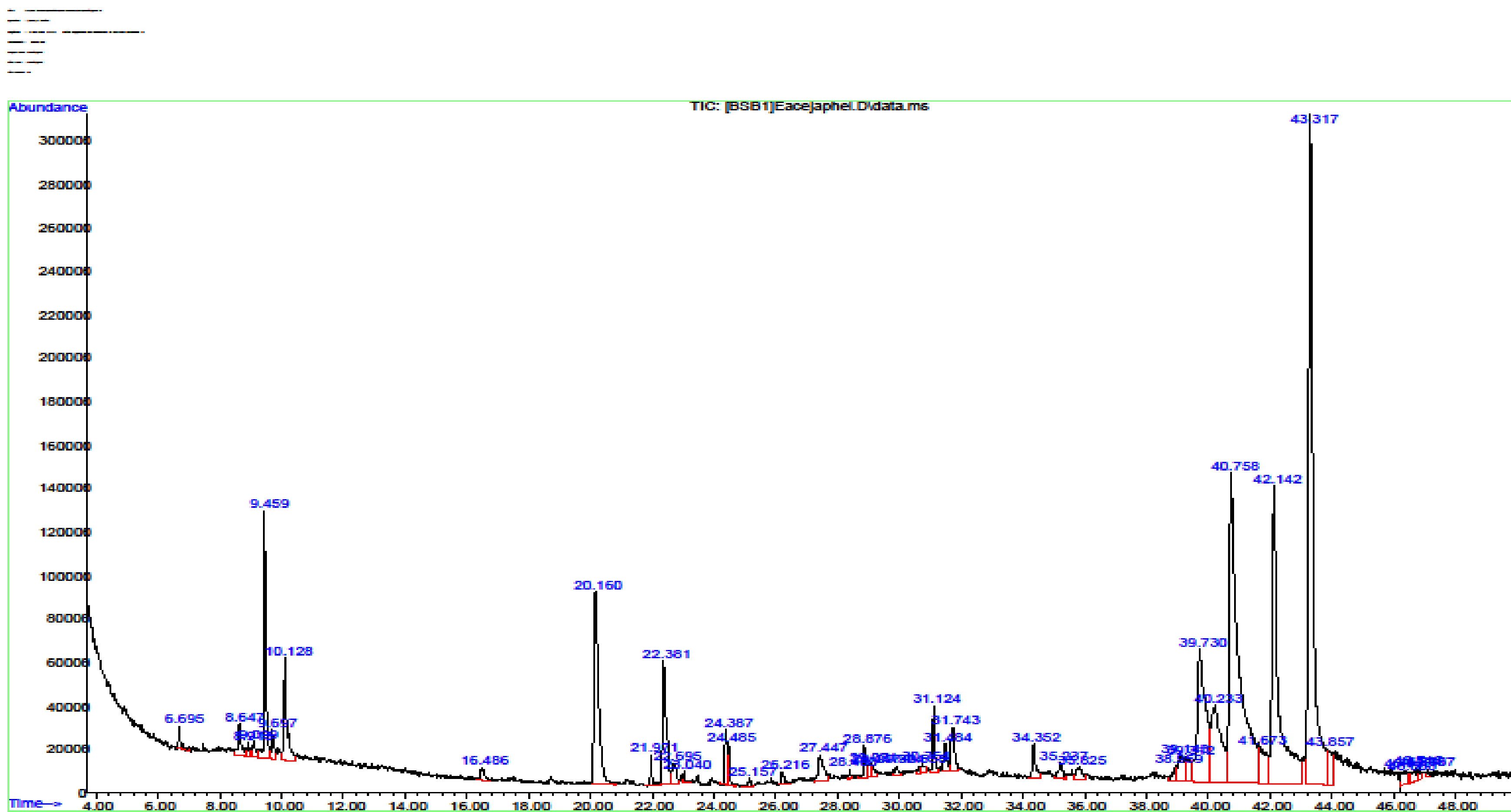


Figure 4.1: A chromatogram of the ethyl aceate leaf extract of *O. basilicum*

4.3 Mineral Elements Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

Table 4.2 shows the mineral elements found in the ethyl acetate leaf extract of *O. basilicum*. Results show that Mg was of the highest concentration in the extract at 1241.60 ± 0.42 mg/100g followed by Ca (1125.10 ± 1.45 mg/100g), K (397.22 ± 1.21 mg/100g), Na (85.35 ± 0.61 mg/100g), Fe (58.30 ± 1.55 mg/100g), Zn (16.40 ± 0.16 mg/100g), P (7.33 ± 0.08 mg/100g), Li (6.73 ± 0.09 mg/100g), Mn (5.41 ± 0.06 mg/100g), Cu (1.25 ± 0.12 mg/100g), Co (0.08 ± 0.02 mg/100g). Two mineral elements, Chromium and Selenium were not detected.

Table 4.2: Mineral Elements Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

Mineral element	Concentration (mg/100g)
Manganese	5.41 ± 0.06
Copper	1.25 ± 0.12
Zinc	16.40 ± 0.16
Magnesium	1241.60 ± 0.42
Calcium	1125.10 ± 1.45
Iron	58.30 ± 1.55
Phosphorous	7.33 ± 0.08
Potassium	397.22 ± 1.21
Sodium	85.35 ± 0.61
Lithium	6.73 ± 0.09
Cobalt	0.08 ±0.02
Selenium	ND
Chromium	ND

Values are expressed as Mean ± S.D for n=3. ND = Not Detected. The detection limit was 0.001 mg/g.

4.4 Heavy Metal Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

Table 4.3 shows the results obtained for heavy metal concentration in the ethyl acetate leaf extract of *O. basilicum*. Among the heavy metals, Cd, Hg, Pb and As were not detected.

Table 4.3: Heavy Metal Concentration in the Ethyl Acetate Leaf Extract of *O. basilicum*

Heavy Metal	Concentration (mg/100g)
Cadmium	ND
Mercury	ND
Lead	ND
Arsenic	ND

ND = Not Detected. The detection limit was 0.001 mg/g.

4.5 *In vitro* Antibacterial Activity of the Ethyl Acetate Leaf Extract of *O. basilicum*

A broad spectrum antibacterial activity was exhibited by the ethyl acetate leaf extract of *O. basilicum*. The reference drug, Gentamycin, had high zones of inhibition that were statistically significant compared to the other tested antibacterial agents. The zones of inhibition of the plant extract ranged from 17.33 ± 0.58 mm to 27.00 ± 2.00 mm in diameter. Among the gram negatives, *P. aeruginosa* had the highest zone of inhibition (27.00 ± 2.00 mm), while among the gram positives, MRSA had the highest zone of inhibition (25.00 ± 1.73 mm) at 1 g/ml concentration of the plant extract. At the same concentration, the *S. aureus* isolate exhibited the lowest zone of inhibition (24.00 ± 1.00

mm) compared to the Gram negative *E. coli* isolate with a 24.68 ± 0.58 mm zone of inhibition.

Quality control strains of bacteria were susceptible to Neomycin while significant resistance was observed among the clinical isolates. There was a gradual decrease in the antibacterial activity of the extract that coincided with a decrease in its concentration. These results are presented in Figure 4.2 and table 4.4.

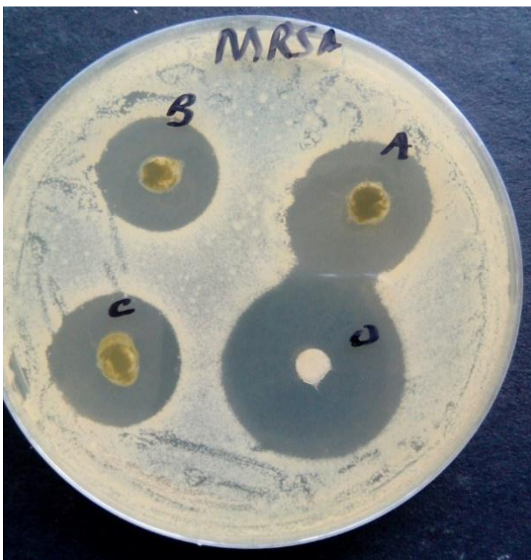


Figure 4.2: Zones of inhibition of Gentamycin and different concentrations of plant extract on MRSA. Key: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D – Gentamycin.

Table 4.4: An Antibiogram of Bacterial Strains

	Zones of Inhibition (mm)						
Test Agents	**MRSA (s)**	***S. aureus* (s)**	***S. aureus* (i)**	***E. coli* (s)**	***E. coli* (i)**	***P. aeruginosa* (s)**	***P. aeruginosa* (i)**
Gentamycin	32.33 ± 2.08^{a}	33.00 ± 1.00^{a}	29.00 ± 1.00^{a}	32.00 ± 1.00^{a}	27.00 ± 1.00^{a}	31.33 ± 1.53^{a}	28.67 ± 1.53^{a}
Neomycin	6.00 ± 00^{d}	27.67 ± 0.58^{b}	13.33 ± 0.58^{e}	31.33 ± 0.58^{a}	15.33 ± 0.58^{e}	27.00 ± 1.00^{b}	14.33 ± 2.08^{c}
DMSO	6.00 ± 0.00^{d}	6.00 ± 0.00^{f}	6.00 ± 0.00^{f}	6.00 ± 0.00^{d}	6.00 ± 0.00^{f}	6.00 ± 0.00^{d}	6.00 ± 0.00^{d}
1g/ml Extract	25.00 ± 1.73^{b}	24.68 ± 0.58^{c}	24.00 ± 1.00^{b}	26.00 ± 1.00^{b}	24.68 ± 0.58^{ab}	27.00 ± 2.00^{b}	26.67 ± 1.53^{a}
0.75g/ml Extract	23.33 ± 1.53^{b}	23.00 ± 1.00^{c}	21.68 ± 1.53^{bc}	24.68 ± 1.53^{b}	22.68 ± 1.53^{bc}	23.33 ± 0.58^{bc}	21.67 ± 1.56^{b}
0.50g/ml Extract	22.00 ± 1.00^{b}	21.68 ± 0.58^{d}	20.33 ± 1.53^{c}	22.67 ± 2.52^{bc}	21.33 ± 1.53^{cd}	22.33 ± 2.52^{c}	21.33 ± 1.53^{b}
0.25g/ml Extract	18.00 ± 0.00^{c}	18.00 ± 1.00^{e}	17.33 ± 0.58^{d}	20.00 ± 1.00^{c}	18.67 ± 1.53^{d}	19.67 ± 0.58^{c}	18.67 ± 1.53^{b}

Values are expressed as Mean ± S.D for n=3. Values with the same superscript letter across columns are not significantly different ($p > 0.05$). Key: s - ATCC strains, i – clinical isolate strains. Susceptibility indices for gentamycin and neomycin ≥ 26 (Susceptible); 23-25 (Intermediate) ≤ 22 (Resistant).

4.5.1: Antibacterial Activity on the *S. aureus* isolate and quality control strain

Gentamycin had the highest antibacterial activity (33.00 ± 1.00 mm) on the quality control *S. aureus* strain while neomycin exhibited the lowest zone of inhibition on the clinical isolate (13.33 ± 0.58 mm). Among the extract concentrations, the 1 g/ml concentration exhibited the highest zone of inhibition (24.68 ± 0.58 mm) on the quality control strain of *S. aureus,* and 24.00 ± 1.00 mm on the clinical isolate. However, this difference was not significant (p = 0.391). The clinical isolate of *S. aureus* exhibited a reduced susceptibility to the organic extract compared to the quality control strain. A notable reduction in the zones of inhibition was observed in tandem with decreasing concentrations of the extract (Figures 4.3a and 4.3b and Table 4.5).

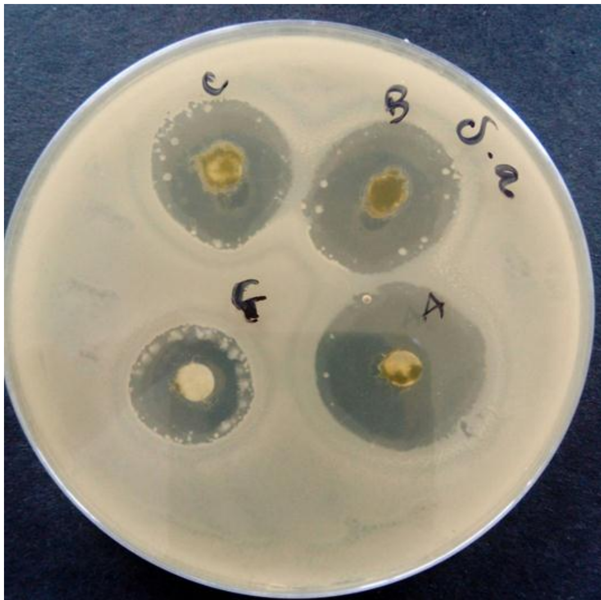


Figure 4.3a: Zones of inhibition of on the ATCC strain of *S. aureus*. Key: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D – Gentamycin.

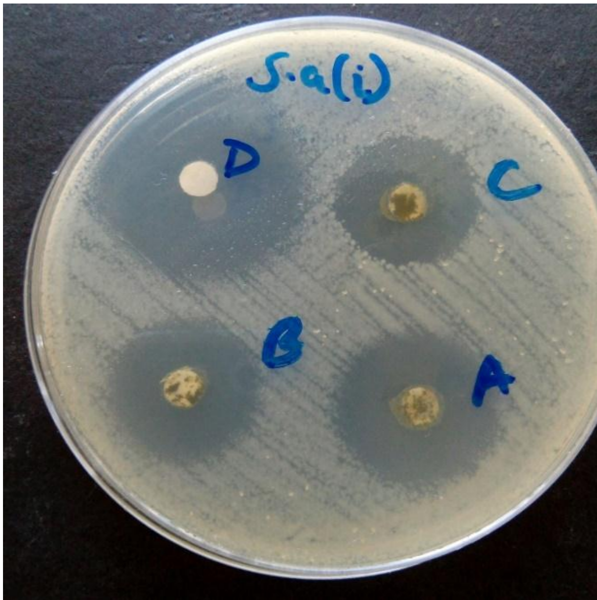


Figure 4.2b: Zones of inhibition of plant extracts on the clinical isolate of *S. aureus*. Key: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D – Gentamycin

Table 4.5: An Antibiogram of *S. aureus*

	Zones of inhibition (mm)		
Test Agents	***S. aureus* (s)**	***S. aureus* (i)**	**p. Values**
Gentamycin	33.00 ± 1.00	29.00 ± 1.00	0.008
Neomycin	27.67 ± 0.58	13.33 ± 0.58	0.000
1g/ml Extract	24.68 ± 0.58	24.00 ± 1.00	0.391
0.75g/ml Extract	23.00 ± 1.00	21.68 ± 1.53	0.295
0.50g/ml Extract	21.68 ± 0.58	20.33 ± 1.53	0.293
0.25g/ml Extract	18.00 ± 1.00	17.33 ± 0.58	0.391

Values are expressed as Mean ± S.D for n=3. Key: s – Quality control ATCC strain, i – clinical isolate strains. Susceptibility indices for gentamycin and neomycin ≥ 26 (Susceptible); 23-25 (Intermediate) ≤ 22 (Resistant).

4.5.2 Antibacterial Activity of the extract on the clinical isolate of *E. coli* and the quality control strain

Gentamycin had a significantly high antibacterial activity on the quality control strain (32.00 ± 1.00mm) compared to the clinical isolate (27.00 ± 1.00mm) (p = 0.04). The clinical isolate of *E. coli* was resistant to neomycin (15.33 ± 0.58 mm). The differences in the antibacterial activities exhibited by the extract between the clinical isolate and the quality control strain was not statistically significant (p>0.05) (Table 4.6).

Table 4.6: An Antibiogram of *E. coli*

	Zones of Inhibition (mm)		
Test Agents	***E. coli* (s)**	***E. coli* (i)**	**p. values**
Gentamycin	32.00 ± 1.00	27.00 ± 1.00	0.004
Neomycin	31.33 ± 0.58	15.33 ± 0.58	0.000
1g/ml Extract	26.00 ± 1.00	24.68 ± 0.58	0.139
0.75g/ml Extract	24.68 ± 1.53	22.68 ± 1.53	0.184
0.50g/ml Extract	22.67 ± 2.52	21.33 ± 1.53	0.490
0.25g/ml Extract	20.00 ± 1.00	18.67 ± 1.53	0.295

Values are expressed as Mean ± S.D for n=3. Key: s – quality control ATCC strain, i – clinical isolate strains. Susceptibility indices for gentamycin and neomycin ≥ 26 (Susceptible); 23-25 (Intermediate) ≤ 22 (Resistant).

4.5.3 Antibacterial Activity on *P. aeruginosa*

Gentamycin had the highest activity on both the quality control strain (31.33 ± 1.53 mm) and the clinical isolate (28.67 ± 1.53 mm) (p=0.099). The clinical isolate of *P. aeruginosa* was resistant to neomycin (14.33 ± 2.08 mm) while the quality control strain

was susceptible (27.00 ± 1.00 mm) (p=0.011). At 1 g/ml concentration, the extract showed the highest antibacterial activity (27.00 ± 2.00 mm) on the quality control strain of *P. aeruginosa,* while at 0.25 g/ml concentration it exhibited the lowest activity (18.67 ± 1.53 mm) on the clinical isolate. Generally, a reduction in the antibacterial activity was observed in tandem with a reduction in the concentration of the extract (Figures 4.4a and 4.4b and Table 4.7).

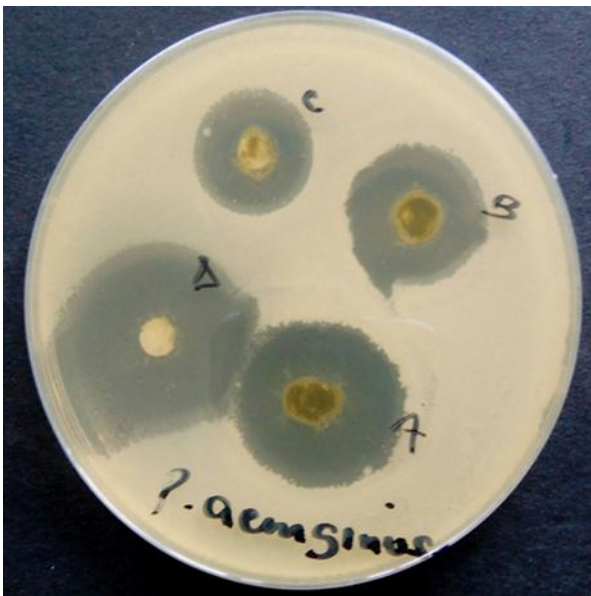


Figure 4.3a: Zones of inhibition on the ATCC strain of *P. aeruginosa.* Key: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D – Gentamycin.

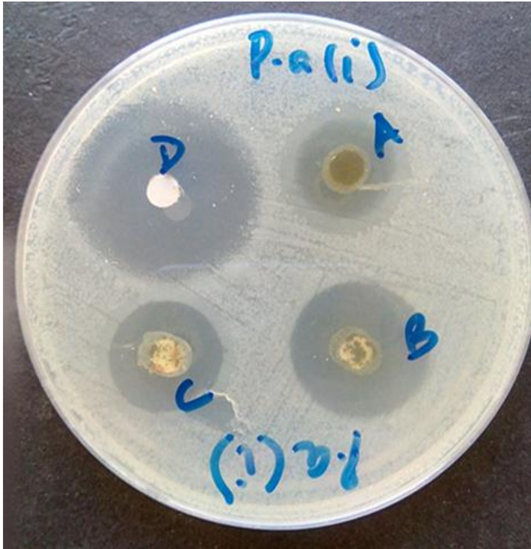


Figure 4.4b: Zones of inhibition on the clinical isolate of *P. aeruginosa.* Figure 4.3a: Zones of inhibition of on the ATCC strain of *S. aureus*. Key: A – 1 g/ml Extract; B – 0.75 g/ml Extract; C – 0.5 g/ml Extract; D – Gentamycin.

Table 4.7: An Antibiogram of *P. aeruginosa*

	Zones of Inhibition (mm)		
Test Agent	***P. aeruginosa* (s)**	***P. aeruginosa* (i)**	**p. values**
Gentamycin	31.33 ± 1.53	28.67 ± 1.53	**0.099**
Neomycin	27.00 ± 1.00	14.33 ± 2.08	**0.011**
1g/ml Extract	27.00 ± 2.00	26.67 ± 1.53	**0.833**
0.75g/ml Extract	23.33 ± 0.58	21.67 ± 1.56	**0.155**
0.50g/ml Extract	22.33 ± 2.52	21.33 ± 1.53	**0.598**
0.25g/ml Extract	19.67 ± 0.58	18.67 ± 1.53	**0.400**

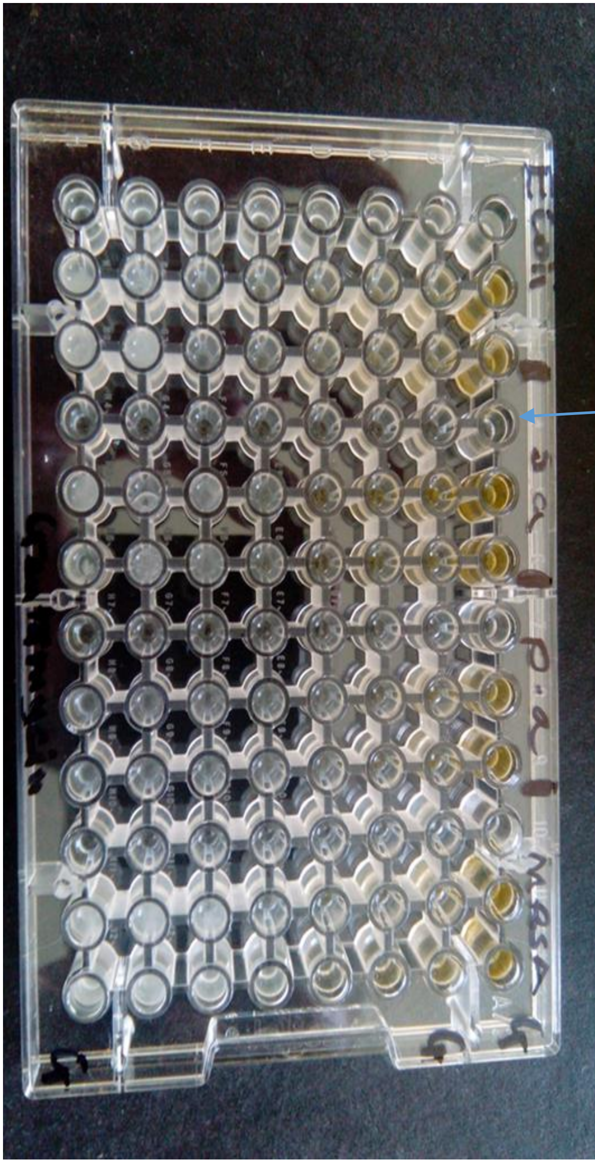
Values are expressed as Mean ± S.D for n=3. Key: s – quality control ATCC strain, i – clinical isolate strains. Susceptibility indices for gentamycin and neomycin ≥ 26 (Susceptible); 23-25 (Intermediate) ≤ 22 (Resistant).

4.5.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The ethyl acetate extract exhibited varied differences in its MIC and MBC value on the different strains of bacteria. Gentamycin had the lowest MIC value (0.156 mcg/ml) for MRSA and both strains of *S. aureus*. Among the Gram negative strains, Gentamycin had an MIC value of 0.312 mcg/ml on both *E. coli* and *P. aeruginosa*. On the quality control strains of *S. aureus*, *E. coli* and *P. aeruginosa*, Neomycin showed an MIC value of 12.5 mcg/ml and an MIC value of 50 mcg/ml on the clinical isolates of the same strains. On *Escherichia coli, S. aureus* and MRSA species, the extract exhibited an MIC of 62.5 mg/ml while on *P. aeruginosa,* it exhibited an MIC of 125 mg/ml.

The MBC of Gentamycin was the lowest, at 0.156 mcg/ml, on the quality control strains of MRSA and *S. aureus* and highest on the clinical isolate of *P. aeruginosa* (1.25 mcg/ml). Neomycin showed an MBC value of 25 mcg/ml on the quality control strain while on the clinical isolate, it showed an MBC value of 100 mcg/ml. The plant extract exhibited an MBC value of 62.5 mg/ml on the *S. aureus* strain and an MBC of 125 mg/ml on MRSA, *E. coli* and *P. aeruginosa* (Figure 4.5 and Table 4.8).

Decreasing concentrations of the antibacterials



Increasing concentrations of the antibacterials

Figure 4.5: A 96 micro-well plate demonstrating MIC value determination

Table 4.8: MIC and MBC Values of Different Antimicrobial Agents on Selected Bacterial Strains

Pathogen	MIC (g/ml)			MBC (g/ml)		
	Gentamycin	**Extract**	**Neomycin**	**Gentamycin**	**Extract**	**Neomycin**
MRSA	0.156	62.5	-	0.156	125.0	-
S. aureus (s)	0.156	62.5	12.5	0.156	62.5	25.0
S. aureus (i)	0.156	62.5	50.0	0.312	62.5	100.0
E. coli (s)	0.312	62.5	12.5	0.312	125.0	25.0
E. coli (i)	0.312	62.5	50.0	0.625	125.0	100.0
P. aeruginosa (s)	0.312	125.0	12.5	0.312	125.0	25.0
P. aeruginosa (i)	0.312	125.0	50.0	1.250	125.0	100.0

Key: s - ATCC strains, i – clinical isolate strains

4.6 Immunomodulatory Potential of Ethyl acetate Leaf Extract of *O. basilicum*

4.6.1 Effect of *O. basilicum* Extract on Cyclophosphamide Induced Myelosuppression

Table 4.9 shows that the ethyl acetate leaf extract of *O. basilicum* exhibited immunomodulatory effects on cyloclophosphamide induced myelossupressed stem cells. On the 1st day, there was no significant difference in the levels of Hb among the different treatment groups while on the 15th day, Hb levels in cyclophosphamide administered mice were significantly lower (6.612 ± 0.430 g/dl) compared to the levels in mice administered with the organic extract of *O. basilicum* at a dose level of 300 mg/kg body weight (12.688 ± 0.831 g/dl). There was no significant difference in the Hb levels among the groups administered with DMSO, levamisole hydrochloride and extract concentrations of 100 and 200 mg/kg body weight ($p>0.05$).

The WBC counts showed no significant difference before the start of the administration period among the different groups. On the 15th day, white blood cell counts were, however, significantly lower in the cyclophosphamide administered group (4.378 ± 0.691 10^3/µl) and significantly elevated in the mice administered with levamisole hydrochloride (10.11 ± 0.652 10^3/µl), this elevation was, however, not statistically different from mice administered with 4% Dimethyl Sulphoxide. The white blood cell counts in mice administered with different doses of the organic extract of *O. basilicum* were not statistically different.

There was no significant difference in the red blood cell counts among the various groups before the start of treatment. On the 15th day, RBC counts in mice administered with cyclophosphamide and extract dose of 100 mg/kg body weight were significantly low (6.304 ± 0.693 10^6/µl and 7.398 ± 0.756 10^6/µl respectively). Mice administered with the extract dose of 300 mg/kg body weight had the highest RBC counts among the different treatment groups on the 15th day (9.182 ± 0.684 10^6/µl). However, total RBC counts in this group were not statistically different from mice administered with the extract dose of 200 mg/kg body weight, levamisole hydrochloride and 4% DMSO.

Table 4.9: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Cyclophosphamide Induced Myelosuppression in Swiss albino Mice

Group	Treatment	Dose	Hematological parameters					
			Hb (g/dL)		WBC (10^3/µl)		RBC (10^6/µl)	
			Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
I	4% DMSO	10 ml/kgbwt	11.356 ± 0.793^{a}	11.182 ± 0.574^{b}	9.698 ± 0.623^{a}	9.622 ± 0.992^{a}	7.836 ± 0.423^{a}	8.214 ± 0.376^{ab}
II	Cyclophosphamide	20 mg/kgbwt	11.684 ± 0.517^{a}	6.612 ± 0.430^{c}	8.884 ± 0.534^{a}	4.378 ± 0.691^{c}	7.712 ± 0.339^{a}	6.304 ± 0.693^{c}
III	Levamisole	50 mg/kgbwt	11.188 ± 0.652^{a}	11.422 ± 0.774^{ab}	9.736 ± 0.858^{a}	10.11 ± 0.652^{a}	7.698 ± 0.604^{a}	8.074 ± 0.658^{ab}
IV	Extract	100 mg/kgbwt	11.658 ± 0.663^{a}	11.284 ± 0.876^{b}	9.306 ± 0.689^{a}	6.852 ± 0.358^{b}	7.712 ± 0.676^{a}	7.398 ± 0.756^{bc}
V	Extract	200 mg/kgbwt	11.436 ± 0.843^{a}	12.068 ± 0.512^{ab}	9.232 ± 0.676^{a}	7.350 ± 0.827^{b}	7.614 ± 0.568^{a}	8.324 ± 0.479^{ab}
VI	Extract	300 mg/kgbwt	11.740 ± 0.582^{a}	12.688 ± 0.831^{a}	8.936 ± 0.955^{a}	8.002 ± 0.356^{b}	7.760 ± 0.465^{a}	9.182 ± 0.684^{a}

Values are expressed as Mean ± S.D for n = 5. Values with the same superscript letters in the same column are not significantly different ($p > 0.05$).

4.6.1.1: Effect of the Extract on Hb levels after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice

Administration of cyclophosphamide to the study mice significantly reduced Hb levels (from 11.684 ± 0.517 g/dL to 6.612 ± 0.430 g/dL, p=0.000). Administration of the extract of *O. basilicum* and the reference drug, levamisole hydrochloride, showed a restoration of Hb levels when compared to the cyclophosphamide administered group. Total Hb regained normal levels on the 15th day of study. This restoration to normalcy was not significant in any of the treatment groups (p>0.05).

Hemoglobin levels were markedly increased in the group administered with the extract dose of 300 mg/kg body weight after the 15th day (12.688 ± 0.831 g/dL). This increase was not significantly different from the levels recorded at the start of the experiment (11.740 ± 0.582 g/dL, p=0.075). The ethyl acetate leaf extract of *O. basilicum,* at a dose of 100 mg/kg body weight, showed lower levels of Hb restoration compared to the other extract concentrations (11.284 ± 0.876 g/dL). The myelosuppressive effect of cyclophosphamide on Hb production was, therefore, overcome by administering mice with the ethyl acetate leaf extract of *O. basilicum*. Increasing concentrations of the leaf extract showed a subsequent improvement in the restoration of Hb levels in a dose dependent manner (Table 4.10).

Table 4.10: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Hemoglobin levels after Cyclophosphamide Induced Myelosuppression in Swiss albino Mice

Group	Treatment	Dose	Hb (g/dL)		p. value
			Day 1	Day 15	
I	4% DMSO	10 ml/kgbwt	11.356 ± 0.793	11.182 ± 0.574	**0.703**
II	Cyclophosphamide	20 mg/kgbwt	11.684 ± 0.517	6.612 ± 0.430	**0.000**
III	Levamisole	50 mg/kgbwt	11.188 ± 0.652	11.422 ± 0.774	**0.621**
IV	Extract	100 mg/kgbwt	11.658 ± 0.663	11.284 ± 0.876	**0.471**
V	Extract	200 mg/kgbwt	11.436 ± 0.843	12.068 ± 0.512	**0.202**
VI	Extract	300 mg/kgbwt	11.740 ± 0.582	12.688 ± 0.831	**0.075**

Values are expressed as Mean ± S.D for n=5. Statistical analysis by 2 – sample T – test ($p \leq 0.05$).

4.6.1.2: Effect of the Extract on Total Leucocyte Counts after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice

Administration of cyclophosphamide to the study mice significantly reduced total leucocyte counts (from 8.884 ± 0.534 to 4.378 ± 0.691 10^3/µl, p=0.000) compared to the control group that was administered with 4% Dimethyl sulphoxide (from 9.698 ± 0.623 to 9.622 ± 0.992 10^3/µl, p=0.889) after a 15 day treatment period. Administration of the organic extract of *O. basilicum* at a dose of 300 mg/kg body weight and the reference drug, levamisole hydrochloride, showed a restoration of leucocyte counts (8.002 ± 0.356 and 10.11 ± 0.652 10^3/µl) comparable to the 1st day. This restoration to normalcy was not significantly different in any of the two treatment groups (p>0.05).

The ethyl acetate leaf extract of *O. basilicum,* at doses of 100 and 200 mg/kg body weight, showed lower levels of leucocyte restoration (6.852 ± 0.358 and 7.350 ± 0.827 10^3/µl). These levels were significantly lower than those recorded on the 1st day in the same treatment group ($p<0.05$). The suppressive effect of the drug, cyclophosphamide, on total leucocyte count was therefore rectified by treating mice with the ethyl acetate leaf extract of *O. basilicum* at a concentration of 300 mg/kg body weight. Increasing the concentration of the leaf extract showed a subsequent increase in the restoration of the leucocyte counts in a dose dependent manner (Table 4.11)

Table 4.11: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on White Blood Cell Counts after Cyclophosphamide Induced Myelosuppression in Swiss albino Mice

Group	Treatment	Dose	WBC (10^3/µl)		p. value
			Day 1	Day 14	
I	4% DMSO	10 ml/kgbwt	9.698 ± 0.623	9.622 ± 0.992	**0.889**
II	Cyclophosphamide	20 mg/kgbwt	8.884 ± 0.534	4.378 ± 0.691	**0.000**
III	Levamisole	50 mg/kgbwt	9.736 ± 0.858	10.11 ± 0.652	**0.463**
IV	Extract	100 mg/kgbwt	9.306 ± 0.689	6.852 ± 0.358	**0.000**
V	Extract	200 mg/kgbwt	9.232 ± 0.676	7.350 ± 0.827	**0.006**
VI	Extract	300 mg/kgbwt	8.936 ± 0.955	8.002 ± 0.356	**0.096**

Values are expressed as Mean ± S.D for n=5. Statistical analysis by 2 – sample T – test ($p \leq 0.05$).

4.6.1.3: Effect of the Extract on Red Blood Cell Count after Cyclophosphamide Induced Myelosuppression in Swiss Albino Mice

Cyclophosphamide significantly reduced RBC levels (from 7.712 ± 0.339 to 6.304 ± 0.693 10^6/μl, p=0.008). The group administered with 4% Dimethyl sulphoxide showed no significant difference in the RBC counts between the 1st day and the 15th day of the treatment period (from 7.836 ± 0.423 to 8.214 ± 0.376 10^6/μl, p=0.008). Restoration of red blood cell counts was observed in mice administered with the extract of *O. basilicum* at doses of 100 mg/kg body weight (7.398 ± 0.756 10^6/μl), 200 mg/kg body weight (8.324 ± 0.479 10^6/μl) and the reference drug, levamisole hydrochloride, (8.074 ± 0.658 10^6/μl). However, these counts were not significantly different from the counts obtained at day 1 (p>0.05).

Red blood cell counts were markedly increased in the group administered with the extract dose of 300 mg/kg body weight after 15 days (9.182 ± 0.684 10^6/μl). This increase in red blood cell count was significantly different from the levels recorded on the 1st day (7.760 ± 0.465 10^6/μl, p=0.006). The suppressive effect of the drug, cyclophosphamide, on red blood cell production was, therefore, overcome by treating mice with the ethyl acetate leaf extract of *O. basilicum*. Increasing the concentration of the leaf extract showed a subsequent improvement in the restoration of red blood cell levels in a dose dependent manner (Table 4.12).

Table 4.12: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Red Blood Cell Counts after Cyclophosphamide Induced Myelosuppression in Swiss albino Mice

Group	Treatment	Dose	RBC (10^6/µl)		p. value
			Day 1	Day 15	
I	4% DMSO	10 ml/kgbwt	7.836 ± 0.423	8.214 ± 0.376	**0.179**
II	Cyclophosphamide	20 mg/kgbwt	7.712 ± 0.339	6.304 ± 0.693	**0.008**
III	Levamisole	50 mg/kgbwt	7.698 ± 0.604	8.074 ± 0.658	**0.378**
IV	Extract	100 mg/kgbwt	7.712 ± 0.676	7.398 ± 0.756	**0.511**
V	Extract	200 mg/kgbwt	7.614 ± 0.568	8.324 ± 0.479	**0.070**
VI	Extract	300 mg/kgbwt	7.760 ± 0.465	9.182 ± 0.684	**0.006**

Values are expressed as Mean ± S.D for n=5. Statistical analysis by 2 – sample T – test ($p \leq 0.05$).

4.6.2 Effect of the Extract on Humoral Antibody Responses to RRBC's in Swiss Albino Mice

All the three doses of the organic extract showed haemagglutination. The group that was administered with cyclophosphamide showed significant inhibition of the hemagglutinin titer (5.6 ± 0.894 HA units/µL) compared to the control group (8.4 ± 0.894 HA units/µL) at $p < 0.05$. Immune modulation of the humoral responses by the extract at a dose of 300 mg/kg body weight resulted in the highest antibody titer (12.4 ± 0.894 HA units/µL) that was significantly different from the other extract doses of 100 mg/kg body weight (8.8 ± 1.095 HA units/µL) and 200 mg/kg body weight (9.6 ± 0.894 HA units/µL) ($p<0.05$). The two doses exhibited a mild potentiation of humoral immunity. The reference drug, levamisole hydrochloride, caused higher antibody titers (11.6 ± 0.894 HA units/µL) that were not significantly different from those caused by the extract at a dose level of 300

mg/kg body weight. A dose dependent increase in hemagglutinin titers was observed in this study (Fig 4.6).

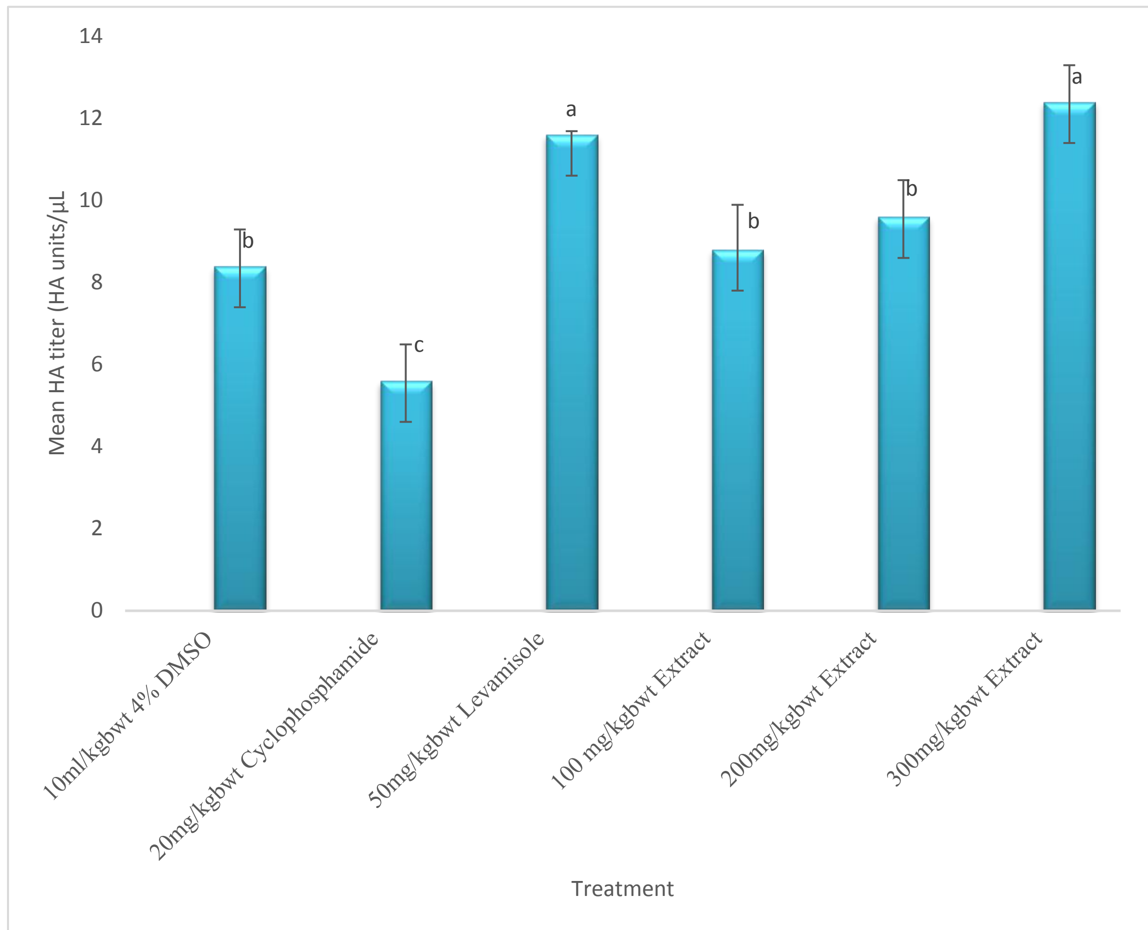


Figure 4.6: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Humoral Antibody Responses to RRBC. Bars with the same letter are not significantly different.

4.6.3: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Neutrophil Adhesion in Swiss Albino Mice

Leucocyte and neutrophil counts were highest in levamisole hydrochloride administered group followed by the group administered with the extract at a dose of 300 mg/kg body weight and, subsequently, the group administered with the extract dose of 200 mg/kg body weight. The same trend was observed in the neutrophil indices in the different treatment groups. In this study, adhesion of neutrophils to nylon fibers was observed to occur in a non-dose dependent manner. The mean % neutrophil adhesion in mice that were administered with the reference drug, levamisole hydrochloride, was higher (13.38 ± 3.35%) than those in the groups that were administered with cyclophosphamide and the plant extracts. However, the difference was not statistically significant.

The highest mean % of neutrophil adhesion was observed in the mice group that was administered with 4% DMSO (15.27 ± 6.66%). At the dose level of 300 mg/kg body weight, the plant extract had the highest mean % neutrophil adhesion (11.084 ± 1.142%) followed by the extract dose of 100 mg/kg body weight (10.58 ± 6.4%). At the dose of 200 mg/kg body weight, the extract exhibited low mean % neutrophil adhesion levels (6.80 ± 2.3%). From the study groups, statistically significant results were not observed ($p>0.05$) (Table 4.13).

Table 4.13: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Neutrophil Adhesion in Swiss Albino Mice

Group	Treatment	Dose	TLC (cells/mm^3) T		Neutrophils (%) N		Neutrophil Index (T*N)		Neutrophil adhesion (%)
			UB	TB	UB	TB	UB	TB	
I	4% DMSO	10 ml/kgbwt	8.882 ± 0.225[de]	8.062 ± 0.306[d]	32.168 ± 1.974[c]	29.926 ± 0.886[d]	285.84 ± 21.46[e]	241.30 ± 12.4[e]	15.27 ± 6.66[a]
II	Cyclophosphamide	20 mg/kgbwt	8.650 ± 0.444[e]	7.906 ± 0.607[d]	30.018 ± 1.129[c]	29.616 ± 1.041[d]	259.85 ± 19.73[e]	234.5 ± 23.4[e]	9.90 ± 3.27[a]
III	Levamisole	50 mg/kgbwt	12.144 ± 0.347[a]	11.442 ± 0.403[a]	46.714 ± 1.304[a]	42.928 ± 1.117[a]	567.4 ± 27.3[a]	491.2 ± 22.9[a]	13.38 ± 3.35[a]
IV	Extract	100 mg/kgbwt	9.692 ± 0.423[cd]	9.3420 ± 0.196[c]	35.932 ± 1.954[b]	33.570 ± 1.312[c]	348.6 ± 29.9[d]	313.60 ± 9.66[d]	10.58 ± 6.4[a]
V	Extract	200 mg/kgbwt	10. 162 ± 0.357[c]	9.692 ± 0.305[bc]	39.016 ± 1.37[b]	38.106 ± 1.148[b]	396.34 ± 15.81[c]	369.50 ± 20.48[c]	6.80 ± 2.3[a]
VI	Extract	300 mg/kgbwt	11. 228 ± 0.632[b]	10.338 ± 0.724[b]	43.650 ± 1.761[a]	42.186 ± 1.915[a]	489.7 ± 26.3[b]	435.6 ± 28.1[b]	11.084 ± 1.142[a]

Values are expressed as Mean ± S.D for n=5. Values with same superscript letters in the same column are not significantly ($p > 0.05$). UB – Nylon fibers untreated blood; TB - Nylon fibers treated blood.

4.6.4: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Mean Foot Paw Edema in DTH Models

This study undertook a functional assessment of *in vivo* effects of the ethyl acetate leaf extracts of *O. basilicum* on cell mediated immunity. As the test entails intradermal antigen inoculation, it was used to assess skin responses that are dependent on antigen specific memory T-cells.

There was a gradual decrease in footpad edema starting at the 8th hour up to the 48th hour. At the 48th hour, increase in mean percentage footpad edema was lowest in the mice group administered with levamisole hydrochloride (7.87 ± 5.00%) followed by the group administered with the plant extract dose of 300 mg/kg body weight (9.40 ± 4.66%). The DMSO administered mice exhibited the highest mean footpad edema at the 48th hour (14.92 ± 3.75%). Table 4.14 and table 4.15 exhibit the different statistical analyses of the collected data.

Table 4.14: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Mean Foot Paw Edema in DTH Models

Group	Treatment	Dose	Mean Foot Paw Edema in %							
			Hour 2	Hour 4	Hour 6	Hour 8	Hour 12	Hour 24	Hour 36	Hour 48
I	4% DMSO	10 ml/kgbwt	27.08 ± 2.15^{a}	27.98 ± 2.19^{b}	35.83 ± 3.14^{a}	36.88 ± 2.42^{a}	30.41 ± 5.38^{a}	27.30 ± 3.07^{a}	21.35 ± 5.03^{a}	14.92 ± 3.75^{ab}
II	Cyclophosphamide	20 mg/kgbwt	27.10 ± 2.04^{a}	36.87 ± 5.26^{ab}	34.35 ± 5.59^{c}	35.03 ± 2.98^{a}	33.67 ± 2.57^{a}	24.23 ± 4.56^{a}	19.16 ± 5.96^{a}	12.15 ± 1.79^{ab}
III	Levamisole	50 mg/kgbwt	31.63 ± 8.22^{a}	38.10 ± 5.30^{a}	47.05 ± 6.01^{ab}	38.13 ± 5.19^{a}	34.50 ± 3.02^{a}	21.13 ± 2.49^{a}	15.58 ± 2.08^{a}	7.87 ± 5.00^{b}
IV	Extract	100 mg/kgbwt	29.40 ± 6.06^{a}	44.25 ± 7.17^{a}	43.20 ± 5.10^{abc}	34.77 ± 3.64^{a}	28.40 ± 5.40^{a}	21.39 ± 4.14^{a}	20.82 ± 4.85^{a}	16.94 ± 5.90^{a}
V	Extract	200 mg/kgbwt	30.59 ± 2.18^{a}	37.56 ± 2.05^{a}	38.15 ± 2.24^{bc}	37.12 ± 4.63^{a}	30.26 ± 6.96^{a}	25.46 ± 1.83^{a}	16.52 ± 5.21^{a}	9.96 ± 2.67^{ab}
VI	Extract	300 mg/kgbwt	27.33 ± 5.69^{a}	41.50 ± 4.82^{a}	47.72 ± 5.61^{a}	39.56 ± 6.33^{a}	33.69 ± 3.33^{a}	27.06 ± 8.71^{a}	20.29 ± 6.83^{a}	9.40 ± 4.66^{ab}

Values are expressed as Mean ± S.D for n=5. Values with the same superscript letter in the same column are not significantly different ($p > 0.05$).

Table 4.15: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Mean Foot Paw Edema in DTH Models

Group	Treatment	Dose	Mean Foot Paw Edema in %							
			Hour 2	Hour 4	Hour 6	Hour 8	Hour 12	Hour 24	Hour 36	Hour 48
I	4% DMSO	10 ml/kgbwt	27.08 ± 2.15[bc]	27.98 ± 2.19[bc]	35.83 ± 3.14[a]	36.88 ± 2.42[a]	30.41 ± 5.38[ab]	27.30 ± 3.07[bc]	21.35 ± 5.03[cd]	14.92 ± 3.75[d]
II	Cyclophosphamide	20 mg/kgbwt	27.10 ± 2.04[bc]	36.87 ± 5.26[a]	34.35 ± 5.59[ab]	35.03 ± 2.98[ab]	33.67 ± 2.57[ab]	24.23 ± 4.56[c]	19.16 ± 5.96[cd]	12.15 ± 1.79[d]
III	Levamisole	50 mg/kgbwt	31.63 ± 8.22[b]	38.10 ± 5.30[ab]	47.05 ± 6.01[a]	38.13 ± 5.19[ab]	34.50 ± 3.02[b]	21.13 ± 2.49[c]	15.58 ± 2.08[cd]	7.87 ± 5.00[de]
IV	Extract	100 mg/kgbwt	29.40 ± 6.06[bc]	44.25 ± 7.17[a]	43.20 ± 5.10[a]	34.77 ± 3.64[ab]	28.40 ± 5.40[bc]	21.39 ± 4.14[cd]	20.82 ± 4.85[cd]	16.94 ±5.90[d]
V	Extract	200 mg/kgbwt	30.59 ± 2.18[abc]	37.56 ± 2.05[ab]	38.15 ± 2.24[a]	37.12 ± 4.63[ab]	30.26 ± 6.96[bc]	25.46 ± 1.83[c]	16.52 ± 5.21[d]	9.96 ± 2.67[de]
VI	Extract	300 mg/kgbwt	27.33 ± 5.69[cd]	41.50 ± 4.82[ab]	47.72 ± 5.61[a]	39.56 ± 6.33[ab]	33.69 ± 3.33[bc]	27.06 ± 8.71[cd]	20.29 ± 6.83[de]	9.40 ± 4.66[ef]

Values are expressed as Mean ± S.D for n=5. Values with the same superscript letter in the same row are not significantly different ($p > 0.05$).

4.6.4.1: Effects of Ethyl acetate extract of *O. basilicum* on Total Leucocyte and Platelet Counts in DTH Models

Administration of the ethyl acetate leaf extract of *O. basilicum* exhibited a dose dependent effect on hematological parameters in delayed type hypersensitivity models. The white blood cell counts were highest in mice administered with levamisole hydrochloride (11.98 ± 1.15 10^3/µl), which was not significantly different from the counts in mice administered with the extract at a dose of 300 mg/kg body weight (9.72 ± 1.70 10^3/µl). These values were significantly different from those recorded in mice administered with cyclophosphamide (6.48 ± 0.70 10^3/µl) only. Even though mice administered with the extract doses of 100, 200 mg/kg body weight and 4% DMSO recorded higher WBC counts than those in the cyclophosphamide administered group, they were not significantly different from each other.

The lymphocyte counts were high in the levamisole hydrochloride administered group (87.14 ± 3.86%) with the percentage count being significantly different from the counts in the cyclophosphamide administered mice (74.76 ± 3.11%) but not significantly different from the counts in other treatment groups. The different doses of the organic leaf extract of *O. basilicum* did not show any significant differences in lymphocyte counts. For the extracts, the percentage of lymphocytes was highest in the 300 mg/kg body weight dose administered group and least in the 100 mg/kg body weight dose administered group (77.78 ± 5.14%).

The granulocytes, as were lymphocyte counts, were highest in the mice administered with levamisole hydrochloride (9.74 ± 1.57%) and least in the group administered with

cyclophosphamide only (6.92 ± 0.46%). The percentage granulocyte levels in levamisole hydrochloride administered mice group were significantly different from those of cyclophosphamide and DMSO administered mice ($p<0.05$) and not significantly different from the mice groups administered with the plant extract ($p>0.05$). The plant extract doses exhibited a dose dependent effect on granulocyte levels with 300 mg/kg body weight dose showing the highest granulocyte concentration (8.16 ± 1.30%) and the 100 mg/kg body weight dose showing the least concentration of granulocytes (7.68 ± 1.19%).

The mean platelet counts were highest in mice administered with the 300 mg/kg body weight dose of the plant extract (317.6 ± 59.6 10^3/µl) with the count being significantly different from the count in cyclophosphamide administered mice group, which showed the lowest counts (201.4 ± 44.0 10^3/µl). Of the three doses used, the 100 mg/kg body weight dose had the lowest platelet count (276.6 ± 52.8 10^3/µl). The plant extract exhibited its effect on platelets in a dose dependent manner.

The percentage PDW was significantly higher in the levamisole hydrochloride and the 300 mg/kg body weight dose administered mice (41.26 ± 2.78% and 40.82 ± 1.10%) compared to the cyclophosphamide administered mice (37.72 ± 1.36%). The % PDW in the two mice groups were not significantly different from the % PDW in the other treatment groups with a gradual reduction in % PDW being observed with a decrease in plant extract concentrations.

The effect of the extract on the plateletcrit in mice was not dose dependent. The highest percentage of plateletcrit was observed in the 100 mg/kg body weight dose administered

mice (0.180 ± 0.045%) followed by the 300 mg/kg body weight dose administered mice (0.178 ± 0.052%). The mice in the two groups had their % PCT being significantly different from the plateletcrit in the cyclophosphamide administered mice (0.096 ± 0.027%).

The P-LCR was also affected in a dose independent manner. The 200 mg/kg body weight dose-administered mice exhibited higher levels of P-LCR (5.66 ± 2.08%) with the cyclophosphamide administered mice having the lowest levels (3.64 ± 0.39%). No significant differences in L-PCR were observed in the various treatment groups (Table 4.16).

Table 4.16: Effects of the Ethyl acetate extract of *O. basilicum* on Total Leucocyte Counts and Platelet Counts in DTH Models

Group	Treatment	Dose	Hematological Parameters							
			TLC			PLT				
			WBC(10^3/µl)	LY (%)	GR (%)	PLT (10^3/µl)	MPV (fL)	PDW (%)	PCT (%)	P-LCR(%)
I	4% DMSO	10 ml/kgbwt	7.34 ± 1.17[c]	78.10 ± 4.74[ab]	7.46 ± 0.85[b]	229.6 ± 40.4[ab]	6.04 ± 0.34[ab]	38.56 ± 1.67[ab]	0.126 ± 0.017[ab]	4.14 ± 0.62[a]
II	Cyclophosphamide	20 mg/kgbwt	6.48 ± 0.70[c]	74.76 ± 3.11[b]	6.92 ± 0.46[b]	201.4 ± 44.0[b]	5.54 ± 0.63[b]	37.72 ± 1.36[b]	0.096 ± 0.027[b]	3.64 ± 0.39[a]
III	Levamisole	50 mg/kgbwt	11.98 ± 1.15[a]	87.14 ± 3.86[a]	9.74 ± 1.57[a]	267.2 ± 25.2[ab]	6.62 ± 0.44[a]	41.26 ± 2.78[a]	0.124 ± 0.030[ab]	3.92 ± 0.48[a]
IV	Extract	100 mg/kgbwt	7.5 ± 0.53[bc]	77.78 ± 5.14[ab]	7.68 ± 1.19[ab]	276.6 ± 52.8[ab]	6.26 ± 0.59[ab]	38.88 ± 0.77[ab]	0.180 ± 0.045[a]	4.46 ± 0.42[a]
V	Extract	200 mg/kgbwt	8.16 ± 0.96[bc]	81.74 ± 5.12[ab]	7.78 ± 0.85[ab]	300.0 ± 47.5[a]	6.36 ± 0.40[ab]	39.12 ± 0.99[ab]	0.168 ± 0.054[ab]	5.66 ± 2.08[a]
VI	Extract	300 mg/kgbwt	9.72 ± 1.70[ab]	81.86 ± 9.19[ab]	8.16 ± 1.30[ab]	317.6 ± 59.6[a]	6.94 ± 0.57[a]	40.82 ± 1.10[a]	0.178 ± 0.052[a]	4.98 ± 1.07[a]

Values are expressed as Mean ± S.D for n=5. Values with same superscript letters in the same column are not significantly different (p>0.05).

4.6.6: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Carbon Clearance Test for Phagocytic Activity in Swiss Albino Mice

Phagocytic index is measured by the rate of removal of carbon particles from the blood stream. In this procedure, the body weights, liver weights and spleen weights of the study mice were not found to be significantly different from each other in all treatment groups. The rate of carbon clearance was highest in the mice administered with levamisole hydrochloride (0.033 ± 0.031) and was found to be significantly different from the rate of carbon clearance in the cyclophosphamide administered mice (0.004 ± 0.002), which recorded the lowest rate ($p<0.05$). In the mice administered with the plant extract, the rate of carbon clearance was highest in the 300 mg/kg body weight dose group (0.015 ± 0.01). These levels were, however, not significantly different from the rates recorded in cyclophosphamide, DMSO and levamisole hydrochloride administered mice groups ($p>0.05$).

The plant extract exhibited a dose dependent activity in phagocytic index. The 300 mg/kg body weight dose caused the highest phagocytic index (0.068 ± 0.048) with the 100 mg/kg body weight dose causing the lowest phagocytic index (0.062 ± 0.047). The reference drug, levamisole hydrochloride, exhibited the highest phagocytic index (0.155 ± 0.146) which was found to be significantly different from the index in the cyclophosphamide administered mice (0.019 ± 0.009) and was not significantly different from the index in the mice administered with the plant extract ($p>0.05$; Table 4.17).

Table 4.17: Effect of the Ethyl acetate Leaf Extract of *O. basilicum* on Carbon Clearance Test for Phagocytic Activity in Swiss Albino Mice

Group	Treatment	Dose	Body wt (mg)	Liver wt (mg)	Spleen wt (mg)	Log OD2	Log OD15	Rate of Carbon clearance (K)	Phagocytic Index
I	4% DMSO	10 ml/kgbwt	28.82 ± 2.060^{a}	1.802 ± 0.319^{a}	0.264 ± 0.046^{a}	-1.106 ± 0.085^{b}	-1.254 ± 0.050^{c}	0.011 ± 0.007^{ab}	0.053 ± 0.027^{ab}
II	Cyclophosphamide	20 mg/kgbwt	28.02 ± 1.899^{a}	1.694 ± 0.186^{a}	0.280 ± 0.036^{a}	-1.077 ± 0.058^{ab}	-1.129 ± 0.062^{abc}	0.004 ± 0.002^{b}	0.019 ± 0.009^{b}
III	Levamisole	50 mg/kgbwt	29.14 ± 1.695^{a}	1.794 ± 0.277^{a}	0.256 ± 0.066^{a}	-0.812 ± 0.398^{ab}	-1.239 ± 0.180^{c}	0.033 ± 0.031^{a}	0.155 ± 0.146^{a}
IV	Extract	100 mg/kgbwt	28.98 ± 1.556^{a}	1.842 ± 0.157^{a}	0.264 ± 0.048^{a}	$- 0.822 \pm 0.538^{ab}$	-1.002 ± 0.156^{ab}	0.014 ± 0.011^{ab}	0.062 ± 0.047^{ab}
V	Extract	200 mg/kgbwt	29.02 ± 1.887^{a}	1.856 ± 0.165^{a}	0.2520 ± 0.034^{a}	-1.040 ± 0.097^{ab}	-1.227 ± 0.082^{bc}	0.014 ± 0.007^{ab}	0.065 ± 0.029^{ab}
VI	Extract	300 mg/kgbwt	28.84 ± 1.683^{a}	1.826 ± 0.215^{a}	0.268 ± 0.019^{a}	-0.726 ± 0.139^{a}	-0.925 ± 0.121^{a}	0.015 ± 0.01^{ab}	0.068 ± 0.048^{ab}

Values are expressed as Mean ± S.D for n=5. Values with same superscript letters in the same column are not significantly different ($p > 0.05$).

4.7 *In vivo* Safety Evaluation of the Ethyl acetate Extract of O. basilicum in Normal Swiss albino Mice Models

4.7.1 Effect on Observatory and Behavioral Indices

The behavior of mice was assessed by general observation from the beginning of the dosing period to the end of the study. The normal mice, having been administered with the ethyl acetate leaf extract of *O. basilicum,* did not exhibit any marked behavioral changes at extract doses of 300, 548 and 1000 mg/kgbw. Normal stability was defined by there being no restlessness, confusion, withdrawal behaviors and other indices (Table 4.18)

Table 4.18: Observatory and Behavioral Indices of Mice Administered with Ethyl Acetate Leaf Extract of *O. basilicum*

Parameter	Observation
Restlessness	None
Confusion	None
Withdrawal behaviors	None
Subcutaneous swellings	Nil
Color and consistency of feces	Normal
Condition of teeth	Normal
Breathing abnormalities	Nil
Gait	Normal
Condition of fur	Normal
Mortality	Nil

4.7.2 Effect of the Ethyl acetate Extract of *O. basilicum* on Absolute Body Weights

The body weights of mice in both control and experimental groups were determined using an electronic precision balance. Increase in weekly body weight from one group to the next was not significantly different. However, there was a significant difference in body weights between the start of dosing and the end of week 4) from week 1 to week 4 (28 days). At the start of the dosing period, mice in the group administered with the extract dose of 548 mg/kg body weight had the highest mean body weight (25.12 ± 3.36g) compared to mice in the other groups, maintaining the same superiority in mean body weight (33.08 ± 1.99g) at the end of the dosing period.

The lowest mean body weight in mice administered with the organic extract was observed in the group administered with the 300 mg/kg body weight dose (31.58 ± 2.42g) after 28 days. The highest change in mean body weight (8.96 ± 1.65g) was recorded in the control group that was administered with 4% Dimethyl sulphoxide while the lowest change (6.62 ± 1.25g) was observed in the group administered with the 300 mg/kg body weight dose. These results are presented in table 4.19.

Table 4.19: Effect of the Ethyl Acetate Leaf Extract of *O. basilicum* on Absolute Body Weights (g) of Mice

Time (Weeks)	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
Acclimatization	23.38 ± 3.26^{c}	24.96 ± 2.04^{c}	25.12 ± 3.36^{c}	24.16 ± 2.75^{c}
Week 1	24.48 ± 3.98^{bc}	26.06 ± 2.51^{bc}	26.68 ± 2.20^{bc}	26.78 ± 2.10^{bc}
Week 2	27.82 ± 3.47^{abc}	28.62 ± 2.86^{abc}	29.54 ± 2.08^{abc}	29.22 ± 2.44^{ab}
Week 3	30.47 ± 3.50^{ab}	30.84 ± 2.97^{ab}	30.98 ± 1.78^{ab}	30.60 ± 2.28^{ab}
Week 4	32.34 ± 3.03^{a}	31.58 ± 2.42^{a}	33.08 ± 1.99^{a}	32.04 ± 1.92^{a}
Δ Change in wt	8.96 ± 1.65	6.62 ± 1.25	7.96 ± 2.99	7.88 ± 2.73

Values are expressed as Mean ± S.D for n=5. Values with the same superscript letter across columns are not significantly different (p>0.05). There was no significant difference along rows.

4.7.3 Effect of the Ethyl acetate Extract of *O. basilicum* on Absolute Organ Weights

At the end of treatment period, mice in experimental and control groups were euthanized using chloroform and their organs excised. Organ weights were measured using an analytical weigh balance. The liver had the highest mean organ weights with a maximum of 1.83 ± 0.36g in the control group and a minimum of 1.55 ± 0.40g in the 1000 mg/kg body weight treatment group. The heart had the lowest mean organ weights with an upper limit of 0.13 ± 0.03g in the 548 mg/kg body weight treatment group and a lower limit of 0.10 ± 0.02g in the 300 mg/kg body weight and 1000 mg/kg body weight treatment groups.

At the end of the treatment period, the brain weighed highest in the control group (0.43 ± 0.03g) and lowest in the 1000 mg/kg body weight treatment group (0.37 ± 0.02g). Most

of the organs had their highest weights in the group administered with 4% DMSO, these include the testes (0.24 ± 0.04g), kidney (0.51 ± 0.09g) and the spleen (0.27 ± 0.05g). The testes weighed least in groups administered with 300 mg/kg body weight and 548 mg/kg body weight (both at 0.21 ± 0.07g), the kidney weighed least in the 300 mg/kg body weight treatment group (0.45 ± 0.13g) while the spleen weighed least in the group administered with 548 mg/kg body weight (0.25 ± 0.05g). Across the different treatment groups, there was no significant difference among the weights of individual organs across the groups (Table 4.20).

Table 4.20: Organ Weights (g) of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

Organ	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
Heart	0.11 ± 0.02	0.10 ± 0.02	0.13 ± 0.03	0.10 ± 0.02
Brain	0.43 ± 0.03	0.38 ± 0.05	0.39 ± 0.04	0.37 ± 0.02
Lungs	0.23 ± 0.05	0.32 ± 0.09	0.28 ± 0.05	0.23 ± 0.04
Testes	0.24 ± 0.04	0.21 ± 0.07	0.21 ± 0.07	0.21 ± 0.08
Kidney	0.51 ± 0.09	0.45 ± 0.13	0.51 ± 0.05	0.48 ± 0.07
Liver	1.83 ± 0.36	1.74 ± 0.30	1.83 ± 0.12	1.55 ± 0.40
Spleen	0.27 ± 0.05	0.26 ± 0.11	0.25 ± 0.05	0.26 ± 0.08

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean organ weights across rows ($p>0.05$).

4.7.4 Effect of the Ethyl acetate Extract of *O. basilicum* on the Relative Organ Weight to Body Weight

This is used to gauge the general health status of an organism. As with individual organ weights, the liver in the control group had the highest mean index (0.0565 ± 0.0092). Various organs had differing organ indices. The heart had its index at a high of 0.0039 ± 0.0010 in the 548 mg/kg body weight treatment group and at a low of 0.0031 ± 0.0007 in the 1000 mg/kg body weight treatment group. The brain (0.0135 ± 0.0007), testes (0.0075 ± 0.0014), kidney (0.0156 ± 0.0019), liver (0.0565 ± 0.0092) and the spleen (0.0085 ± 0.0024) all had their organ indices high in the group administered with DMSO.

Most of the organs exhibited low index levels in the groups administered with the ethyl acetate leaf extract of *O. basilicum*. These include the brain (0.0116 ± 0.0006), the lungs (0.0072 ± 0.0011) and the liver (0.0482 ± 0.0106) that had low indices in the 1000 mg/kg body weight treatment group. The testes (0.0064 ± 0.0019) and the spleen (0.0076 ± 0.0016) had low indices in the 548 mg/kg body weight treatment group while the kidney had a low index (0.0144 ± 0.0041) in the 300 mg/kg body weight treatment group. There was no significant difference in the relative organ weight to body weight among the various treatment groups (Table 4.21).

Table 4.21: Relative Organ Weight to Body Weight Ratios of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

Organ	Control	300mg/kgbw	548mg/kgbw	1000mg/kgbw
Heart	0.0034 ± 0.0004	0.0032 ± 0.0006	0.0039 ± 0.0010	0.0031 ± 0.0007
Brain	0.0135 ± 0.0007	0.0124 ± 0.0014	0.0117 ± 0.0013	0.0116 ± 0.0006
Lungs	0.0072 ± 0.0015	0.0100 ± 0.0030	0.0085 ± 0.0015	0.0072 ± 0.0011
Testes	0.0075 ± 0.0014	0.0066 ± 0.0023	0.0064 ± 0.0019	0.0066 ± 0.0024
Kidney	0.0156 ± 0.0019	0.0144 ± 0.0041	0.0154 ± 0.0005	0.0150 ± 0.0018
Liver	0.0565 ± 0.0092	0.0550 ± 0.0092	0.0555 ± 0.0025	0.0482 ± 0.0106
Spleen	0.0085 ± 0.0024	0.0081 ± 0.0032	0.0076 ± 0.0016	0.0079 ± 0.0022

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean organ indexes across rows (p >0.05).

4.7.5 Effect of the Ethyl acetate Extract of *O. basilicum* on Hematological Parameters

4.7.5.1 Red Blood Cell Indices

The mean RBC, HB, RDW and HCT counts were found to be elevated in the mice administered with the extract dose of 1000 mg/kg body weight. The MCHC and MCV values were elevated in the control group. The MCHC was elevated in the group administered with 300 mg/kg body weight (26.60 ± 1.85g/dL).

Low concentrations of various red blood cell indices were recorded in the 300 mg/kg body weight treatment group. These include RBC (7.36 ± 0.7810^6/µl), Hb (9.96 ± 1.15),

MCH (13.54 ± 1.04Pg), MCV (51.00 ± 2.37fL) and HCT (37.58 ± 4.50%). The MCHC was low in the 548 mg/kg body weight treatment group (25.20 ± 1.78 g/dL). These differences were not dose dependent, were not statistically significant and were small in magnitude of change with respect to the controls. These variations in RBC indices were considered to have occurred sporadically without biological or toxicological significance. These results are presented in table 4.22.

Table 4.22: Red Blood Cell Indices of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

RBC indices	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
RBC(10^6/μl)	7.78 ± 0.60	7.36 ± 0.78	7.54 ± 0.96	8.04 ± 0.19
HB (g/dL)	11.16 ± 0.87	9.96 ± 1.15	10.42 ± 0.99	11.26 ± 0.56
MCH (Pg)	14.38 ± 0.97	13.54 ± 1.04	13.90 ± 1.22	14.00 ± 0.80
MCV(fL)	55.10 ± 3.95	51.00 ± 2.37	53.40 ± 3.27	54.16 ± 3.48
MCHC (g/dL)	26.00 ± 0.86	26.60 ± 1.85	25.20 ± 1.78	25.92 ± 1.89
RDW (%)	16.70 ± 2.56	18.04 ± 1.84	18.52 ± 3.13	18.52 ± 3.13
HCT (%)	42.80 ± 3.84	37.58 ± 4.50	41.38 ± 2.98	43.58 ± 3.26

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean red blood cell indices across rows (p>0.05).

4.7.5.2 White Blood Cell Indices

The Mean WBCs (10.26 ± 3.20 10^3/μl) and LY (78.92 ± 4.18%) counts were elevated in the control group and low in the group administered with the extract dose of 548 mg/kg body weight (8.34 ± 3.11 10^3/μl and 72.28 ± 4.06% respectively). The Mean GR levels were elevated in the 548 mg/kg body weight treatment group (20.78 ± 4.06%) and

low in the 1000 mg/kg body weight treatment group (13.96 ± 4.72%). Mean MO levels were low in the control group (6.44 ± 1.80%) and high in the 548 mg/kg body weight treatment group (8.94 ± 1.38%). Compared to their respective controls, statistical analysis of WBC data did not detect a significant increase or drop in the mean red blood cell and white blood cell indices in treatment groups compared with the control. The transient increase or decrease in the different indices were not dose dependent and, therefore, they were not considered to be toxicologically relevant because of their low magnitude. These results are presented in table 4.23.

Table 4.23: White Blood Cell Indices of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

WBC indices	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
WBC (10^3/µl)	10.26 ± 3.20	9.66 ± 2.38	8.34 ± 3.11	9.18 ± 3.92
LY (%)	78.92 ± 4.18	76.82 ± 3.39	72.28 ± 4.06	78.88 ± 4.23
GR (%)	14.84 ± 4.60	15.46 ± 2.59	20.78 ± 4.06	13.96 ± 4.72
MO (%)	6.44 ± 1.80	7.72 ± 1.93	8.94 ± 1.38	6.54 ± 2.03

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean white blood cell indices across rows ($p > 0.05$).

4.7.5.3 Platelet Indices

The Mean PLT and PCT counts were elevated in the control group (717.20 ± 93.30 10^3/µl and 0.33 ± 0.05% respectively) and low in the 300 mg/kg body weight treatment group (629.80 ± 78.8010^3/µl and 0.21 ± 0.09% respectively). The Mean MPV were elevated in the 300 mg/kg body weight treatment group (4.78 ± 0.94fL) and low in the

1000 mg/kg body weight treatment group (4.10 ± 0.52fL). The Platelet distribution width was high in the 548 mg/kg body weight treatment group (16.98 ± 1.46%) and low in the 300 mg/kg body weight treatment group (16.68 ± 0.74%). There was a significant difference between the mean platelet counts in the 1000 mg/kg body weight treatment group and the control group. The other mean platelet indices did not show any significant differences among the groups (Table 4.24).

Table 4.24: Platelet Indices of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

Platelet Indices	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
PLT (10^3/µl)	717.20 ± 93.30^{a}	629.80 ± 78.80^{ab}	643.40 ± 83.50^{ab}	556.00 ± 76.00^{b}
MPV (fL)	4.58 ± 0.38^{a}	4.78 ± 0.94^{a}	4.38 ± 0.52^{a}	4.10 ± 0.52^{a}
PDW (%)	16.88 ± 0.72^{a}	16.68 ± 0.74^{a}	16.98 ± 1.46^{a}	16.86 ± 0.74^{a}
PCT (%)	0.33 ± 0.05^{a}	0.21 ± 0.09^{a}	0.28 ± 0.04^{a}	0.26 ± 0.15^{a}

Values are expressed as Mean ± S.D for n=5. Values with same superscript letters in the same row are not significantly different (p > 0.05).

4.7.6 Biochemical Parameters

4.7.6.1 Liver Function Markers

Liver function levels are used as biomarkers for hepatocellular damage. Alanine transaminase levels were high in the 1000 mg/kg body weight treatment group (66.98 ± 3.97 U/L) and low in the control group (62.12 ± 1.39 U/L). However, these mean differences were not significant. No significant difference was observed in mean AST levels, which were high in the 1000 mg/kg body weight treatment group (198.64 ± 10.50 U/L) and low in the 300 mg/kg body weight treatment group (179.88 ± 8.14 U/L). Mean

ALP levels were high in the 1000 mg/kg body weight treatment group (16.40 ± 2.88 U/L) and low in the control group (12.00 ± 2.45 U/L). Albumin was high in the 1000 mg/kg body weight treatment group (18.44 ± 2.71 g/l) and low in the 548 mg/kg body weight treatment group (13.22 ± 4.26 g/l). Total bilirubin levels were elevated in the 300 mg/kg body weight treatment group (8.54 ± 2.24 μmol/L) and low in the 548 mg/kg body weight treatment group (7.24 ± 1.58 μmol/L).

Direct bilirubin and TP were high in the control groups (6.10 ± 1.48 μmol/L and 42.50 ± 2.50 g/l respectively), and low in the 548 mg/kg body weight treatment groups (4.16 ± 0.68 μmol/L and 39.40 ± 1.01 g/l). Triglyceraldehydes and TC were high in the control group (1.30 ± 0.26 mmol/L and 2.04 ± 0.23 mmol/L respectively) and low in the 300 mg/kg body weight treatment group (0.96 ± 0.15 mmol/L and 1.52 ± 0.13 mmol/L respectively) (Table 4.25).

Table 4.25: Liver Function Levels of Mice Administered with the Ethyl Acetate Leaf Extract *O. basilicum*

Parameter	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
ALT (U/L)	62.12 ± 1.39	62.78 ± 4.70	64.10 ± 4.90	66.98 ± 3.97
AST (U/L)	180.65 ± 15.28	179.88 ± 8.14	183.02 ± 8.47	198.64 ± 10.50
ALP (U/L)	12.00 ± 2.45	13.00 ± 2.92	15.00 ± 1.58	16.40 ± 2.88
ALB (g/l)	15.56 ± 2.38	14.30 ± 2.71	13.22 ± 4.26	18.44 ± 2.71
TBIL (μmol/L)	8.40 ± 2.05	8.54 ± 2.24	7.24 ± 1.58	8.28 ± 2.57
DBIL (μmol/L)	6.10 ± 1.48	4.60 ± 1.55	4.16 ± 0.68	4.52 ± 0.62
TP (g/l)	42.50 ± 2.50	41.86 ± 1.83	39.40 ± 1.01	42.10 ± 2.91
TG (mmol/L)	1.30 ± 0.26	0.96 ± 0.15	1.00 ± 0.33	1.10 ± 0.20
TC (mmol/L)	2.04 ± 0.23	1.52 ± 0.13	1.74 ± 0.20	1.82 ± 0.55

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean liver function levels across rows by One Way ANOVA (p>0.05).

4.7.6.2 Renal Function Markers

To determine the integrity of the kidney after the treatment period, blood urea and creatinine levels were measured. There was no significant differences in the mean urea and creatinine levels across the different groups (p>0.05). Mean urea and creatinine levels were elevated in the 300 mg/kg body weight treatment group (4.21 ± 1.27mmol/L and 127.54 ± 15.38µmol/L respectively). Urea levels were low in the 548 mg/kg body weight treatment group (3.11 ± 0.18mmol) while creatinine levels were low in the 1000 mg/kg body weight treatment group (107.78 ± 4.97µmol/l) (Table 4.26).

Table 4.26: Renal Function Levels of Mice Administered with the Ethyl Acetate Leaf Extract of *O. basilicum*

Parameter	Control	300 mg/kgbw	548 mg/kgbw	1000 mg/kgbw
UREA	4.06 ± 1.05	4.21 ± 1.27	3.11 ± 0.18	3.33 ± 0.32
CRE	111.38 ± 11.06	127.54 ± 15.38	110.66 ± 9.76	107.78 ± 4.97

Values are expressed as Mean ± S.D for n=5. There was no significant difference in mean renal function levels across rows by One Way ANOVA (p >0.05).

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Phytochemical Composition of the Ethyl acetate Extract of *O. basilicum*

Phytochemical constituents and their identification vary within the same species of plants depending on the part of the plant material used, the geographical distribution of the plant, the time of collection and extraction variables (time, temperature, particle size and concentration) (Ghasemzadeh *et al.,* 2018). The phytochemical profiles of the ethyl acetate leaf extract of *O. basilicum* were identified using GC-MS. A total of 30 compounds were identified. The gas chromatogram revealed concentrations of various compounds that were eluted at different retention times. These compounds were identified from the data library of the National Institute of Standards and Technology. Among the compounds identified include terpenoids, phenols, quinolones, esters, essential oils, amphetamines, aldehydes, flavonoids and amines.

Flavonoids are widely distributed among plants. They are free radical scavengers and water soluble antioxidants with the ability to prevent oxidative cell damage. They are active against cancer and protect against all stages of carcinogenesis. These plant metabolites are important in protecting the body against inflammation, allergens,

microbes, platelet aggregation, tumors, viruses and hepatoxins (Farquhar 1996; Natarajan *et al.,* 1999; Ayyanar and Iganacimutha, 2005; Hertog *et al.,* 2007). The flavonoid 6,3'-Dimethoxyflavone was identified in the ethyl acetate leaf extract of *Ocimum basilicum*. Methylated flavonoids have been known to exhibit antimicrobial activities. A derivative of dimethoxyflavone has been established to have a broad spectrum antibacterial activity against *E. coli, S. aureus* and *P. aeruginosa* (Teffo *et al.,* 2010; Omosa *et al.,* 2014).

Also present in the extract were two alkaloids; 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester and Benzo[h]quinolone, 2,4-dimethyl-. Benzo[*h*]quinolones have been used to obtain novel compounds that possess antibacterial activities on *S. aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes*. These compounds are also strong antioxidants and show significant wound healing activities. They protect from oxidative DNA damage caused by harmful free radical reactions (Naik *et al.,* 2007). Pachkore and Dhale (2012) recorded high amounts of alkaloids in the leaf extracts of *O. basilicum* (47 mg/100g). While determining the qualitative phytochemical composition of *Ocimum gratissimum (O. gratissimum)* and *Ocimum sanctum (O. sanctum)* respectively, Agarwal & Varma (2014) and Singh *et al.* (2013) reported the presense of alkaloids. Talabi and Makanjuola (2017), in their quantitative phytochemical screening of *O. gratissimum,* documented that alkaloids and saponins were the most abundant at 0.2875% and 0.225% respectively. Synthetic derivatives of alkaloids are medicinally important for their antispasmodic, analgesic and bactericidal effects. Its physiological activities are apparent when this plant metabolite is administered in animal models (Stray, 1998).

Terpenoids represent the most diverse and largest class of chemicals among the many metabolites produced by plants. They are a group of compounds possessing an isoprene unit as their basic structure. Their classification include sesquiterpenes, monoterpenes and diterpenes among others, based on the number of carbon atoms. Terpenoids play important roles in plants as metabolites, growth hormones and as photosynthetic pigments (Bartley and Scolnick, 1995; Hong *et al.,* 2013; Tholl 2015). They have also been shown to provide protection against pathogenic microorganisms (Yadav *et al.,* 2015). Furthermore, they exhibit a varied range of properties including antitumor effects (Chen *et al.,* 2015), antioxidant activities (Zhang *et al.,* 2017), and osteogenic action (Deepak *et al.,* 2015).

Nootkatone is a sesquiterpene that is synthesized by the oxidation of valencene (Leonhardt and Berger, 2015). It has various biological activities such as antioxidation effects on diesel exhaust particles in the lungs and antifibrotic effects on carbon tetrachloride-induced tumor necrosis of the liver (Kurdi *et al.,* 2018; Nemmar *et al.,* 2018). This compound has also been shown to exhibit antibacterial activities against Gram-positive bacteria including *S. aureus*, *Enterococcus faecalis*, *Corynebacterium diphtheriae, Listeria monocytogenes* and *Bacillus cereus* (Yamaguchi, 2019). *β*-caryophyllene is a sesquiterpene that has been isolated from several plants including *Ocimum basilicum.* It is a component of dietary regimes that is consumed as a food preservative, flavour or additive and is often referred to as a 'dietary cannabinoid' (Gertsch *et al.,* 2008). It is an ingredient of confectionaries, toothpastes, candies, chewing

gums, pharmaceuticals, beverages and cosmetic products (Bhatia *et al.,* 2008; de Groot *et al.,* 2015). *β*-caryophyllene exhibits therapeutic potential due to its multiple pharmacological properties such as antioxidant, anti-inflammatory (Gertsch *et al.,* 2008), antimicrobial (Donati *et al.,* 2015), anticancer and chemopreventive (Sain *et al.,* 2014).
A review by Francomano *et al.,* (2019) showed that *β*-caryophyllene has a variety of biological functions, including the exertion of anti-inflammatory actions by inhibiting inflammatory mediators such as inducible nitric oxide synthase, IL-1β, IL-6 and TNF-α. It has also been shown that *β*-caryophyllene is useful against *Streptococcus* infections, steatohepatitis, osteoporosis and exerts anticonvulsant, myorelaxing, analgesic, sedative, and antidepressive effects. Furthermore, it is established that *β*-caryophyllene possesses strong antimicrobial activities (Xiong *et al.,* 2013). Dahham *et al.* (2015) and Schmidt *et al.* (2006) documented that *β*-caryophyllene has antibacterial and pronounced antifungal effects (Schmidt *et al.,* 2006; Dahham *et al.,* 2015).

The sesquiterpene zierone was also identified in the leaf extract. Zierone has been previously identified in the leaf oil of *Melicope denhamii,* which was reported to have antibacterial activities on *Bacillus subtilis* and *E. coli* (George *et al.,* 2015).

Triterpenoids are plant constituents with a great pharmacological potential. They possess therapeutic activities, including anticancer, antiviral, anti-inflammatory, antibacterial, antidiuretic, antifungal, giardicidal, and can act as acetylcholinesterase inhibitors (Bandeira *et al.,* 2002; Melo *et al.,* 2010; Matos *et al.,* 2013). Nogueira (2018) reviews

that *β*-Amyrin is a pentacyclic triterpene that exerts its anti-inflammatory activities by attenuating L- arginine- induced proliferation in pancreatic wet weight/body weight ratio and by decreasing serum levels of amylase lipase, IL-6 and TNF- α. It also suppresses pancreatic edema, acinar cell necrosis and inflammatory cell infiltration. This compound has also been shown to be gastroprotective, hepatoprotective, antihyperglycemic and hypolipidemic.

The GC-MS analysis also revealed the presence of lupeol acetate, a pentacyclic triterpene. It is a molecule that exhibits a wide spectrum of pharmacological effects on various acute or chronic diseases, including but not limited to arthritis, diabetes, renal disorders, hepatotoxicity, cancer, cardiovascular disease, antinociceptive, anti-inflammatory and microbial infections (Sudhahar *et al.,* 2008; Chen *et al.,* 2012; Alqahtani *et al.,* 2013; Yokoe *et al.,* 2015; Badshah *et al.,* 2016).

Studies have shown the importance of triterpenes obtained from medicinal plants. These compounds improve the quality of healing through mechanisms that range from regulation of pro and anti-inflammatory mediators, growth factors and chemokines (Liu *et al.,* 2008; Sharath *et al.,* 2010; Kim *et al.,* 2013; Chen *et al.,* 2013). The triterpene 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene was also eluted from the ethyl acetate leaf extract of *Ocimum basilicum*. Manool oxide, a diterpene was established to be present in the plant. Manool oxide is found in abundance in several essential oils, from

several plants, which have been shown to have antibacterial effects (Amri *et al.,* 2017; Rosato *et al.,* 2018).

Terpenoids have been reported to have potential therapeutic implications in inflammation (Las Heras *et al.,* 2003). With varying concentrations, different accessions of *Ocimum* have been reported to contain terpenoids (Vimala *et al.,* 2014; Tantry *et al.,* 2016; Akinmoladun *et al.,* 2016). However, in a species of *O. canum*, Aluko *et al.* (2012) reported that terpenoids could not be detected.

Fatty acids including Oxirane, 2-methyl-2-(1-methylethyl)-; 11-Eicosenoic acid, methyl ester and 1,3-Dimethyl-5-isobutylcyclohexane were found to be present in the extract. 11-Eicosenoic acid, a methyl ester, has been associated with antibacterial activities on various pathogens (Suresh *et al.,* 2014). 11-Eicosenoic acid has also been shown to have anti-inflammatory effects (Pereira *et al.,* 2014). The presence of different amines in the ethyl acetate leaf extract of *O. basilicum* were established. These included 9-Thiabicyclo [3.3.1] non-6-en-2-amine, N-methyl-, endo-; 1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl) amino- and 2-Methoxyamphetamine. Bicyclic amines have been documented to have antimicrobial and antiparasitic effects (Weis & Seebacher, 2009; R0ig-Molina *et al.,* 2018).

Aldehydes found in the extract upon GCMS analysis included E-11(13-Methyl) tetradecen-1-ol acetate, Tridecenol<2E-> and tetradecanal. These are long-chain fatty

alcohols that are known to exhibit antimicrobial activity. The potency of long chain alcohols as antimicrobial agents is most mostly evident when the carbon chain length ranges from 10 to 13. Tridecanol and tetradecanal have both been shown to have antibacterial activities on *S. aureus* (Togashi *et al.,* 2007).

Different phenolic compounds were detected in the ethyl acetate leaf extract of *O. basilicum*. The included 1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl-; 3,6,6-Trimethyl-cyclohex-2-enol and l-Alanine, N-(2-thienylacetyl)-, butyl ester. Compounds containing imidazole moieties possess potent antibacterial properties (Sun *et al.,* 2018). The presence of the imidazole ring to the quinolone moiety increases its antibacterial activity (Sun *et al.,* 2018).

5.1.2 Essential and Trace Mineral Element Contents of Ethyl acetate Extract of *O. basilicum*

Heavy metal, mineral element, micronutrient and trace element levels in plants is dependent on the chemical and physical properties of the soil such as the presence of organic matter and pH, stage of growth of the plant and the ability of plants to accumulate some of these elements. Other causes of variations include rainfall, agricultural and industrial practices and temperature (Kawashima & Valente-Soares, 2003; Demirel *et al.,* 2008; Lisiewska *et al.,* 2009).

Major minerals are required in quantities above 100 mg per day and comprise 1% or less of the body weight. They include Ca, Mg, K, Cl and Na. They function in cellular and

basal metabolism, are components of tissues and are important in acid-base balance. Trace elements are required in low quantities of less than 100 mg per day. These elements make up less than 0.01% of the body weight. They include Zn, Fe, Mn, Cu and Cr. Trace elements are very important in the normal functioning of the body as they are key components of proteins including hemoglobin and hemoprotein (Rahmatollah and Mahbobeh, 2010).

These elements play vital roles in enzyme systems and biochemical functions even in low doses. Trace elements are a significant health hazard for man and are an area of concern and priority in research (Imelouane *et al.,* 2011). Imelouane *et al.* (2011) in their review, state that studies have shown that elements such as K, Na, Ca, Mg, Cu and Zn reduce individual risk factors related to cardiovascular diseases of both animals and human beings.

In this study, it was observed that Mg was the element with the highest concentration (1241.6 ± 0.42 mg/100g) in the *O. basilicum* extract. The recommended dietary allowance for Mg in human males and females has been established to be 420 and 320 mg per day respectively. Magnesium is a divalent cation found in abundance intracellularly. It is essential as a cofactor in a variety of enzymatic reactions that are important in the generation of energy from adenosine triphosphate (ATP) and for physiologic processes such as the maintenance of cardiovascular tone and neuromuscular functions (Saris *et al.,* 2000). Deficiency in Mg can, therefore, lead to cardiac

malfunction and muscle weakness (IOM, 1997). Sanni *et al.* (2008) and Pachkore and Dhale (2012) documented low levels of Mg in *O. basilicum*. Magnesium has been quantified in different accessions of Ocimum, even in trace qauantities (Asaolu *et al.,* 2012; Pachkore and Dhale, 2012; Kiendrebeogo *et al.,* 2016; Vidhani *et al.,* 2016).

Calcium is the most important element in bone formation and normal nerve and muscle functioning (IOM, 1997). This element was found to be in high abundance in the *O. basilicum* extract (1125.1 ± 1.45 mg/100g). The recommended dietary allowance for human males and females aged between 31 to 50 is 1000 mg/day (IOM, 1997). This result is in tandem with other studies that found similar levels of quantifiable calcium levels in different accessions of Ocimum (Sanni *et al.,* 2008; Kiendrebeogo *et al.,* 2016). Potassium is an important mineral element functioning as a diuretic and is found in abundance in the leafy materials because it is an activator of some enzymes, such as photosynthetic enzymes. It is an intracellular cation that regulates cellular osmotic balance (IOM, 2005; Antoine, 2012). Potassium had a concentration of 397.22 ± 1.21 mg/100g in the *O. basilicum* extract. Adequate K intake for human males and females aged 31-50 is 4700 mg/day (IOM, 2005). It has been quantified in different Ocimum species (Sanni *et al.,* 2008; Kiendrebeogo *et al.,* 2016). High concentrations of K can, however, interfere with Na absorption, retention and Mg utilization in the body (Imelouane *et al.,* 2011).

Sodium and potassium are important elements in maintaining the ionic balance of the body and in tissue excitability. Sodium is also essential in metabolite transport because of its solubility (Indrayan *et al.,* 2005). Sodium was found in low quantities (85.35 ± 0.61 mg/100g) compared to K in *O. basilicum* extract. This is because the uptake of Na is normally via the young root tips in which the endodermis cell walls are unsupervised (Mengel and Kirkby, 1987). Adequate Na intake per day is 1500 mg (IOM, 2005). As with the other mineral elements, Sodium has been documented in both *O. basilicum* and other species of the same genus (Daniel *et al.,* 2011; Pachkore and Dhale 2012).

Iron is a vital component of hemoglobin and helps in oxygen transportation. Together with hemoglobin and ferredoxin, iron plays an important role in metabolism. Excess iron intake has been associated with dizziness, vomiting, nausea, diarrhea, shock, joint pain and liver damage. Excess iron has toxic effects on metabolic functions and on the cardiovascular system (Martin & Griswold, 09). The ethyl acetate extract of *O. basilicum* was found to have an Iron concentration of 58.3 ± 1.55 mg/100g. The WHO permissible levels of iron in medicinal plants has not yet been established, however, the recommended dietary iron allowance for human males and females is 8 and 18 mg/day respectively (IOM, 2001). Presence of Iron has been reported in *O. basilicum* and other species of Ocimum (Oboh *et al.,* 2009; Pachkore and Dhale, 2012; Kiendrebeogo *et al.,* 2016).

Other mineral elements found in the organic extract of *O. basilicum* include Zinc (16.4 ± 0.16 mg/100g) which is associated with different enzymes especially those involved in ribonucleic acid (RNA) synthesis. The WHO/ FAO permissible limits for Zinc in herbal products is 50 mg/kg (WHO, 2005; WHO, 2006), while the recommended daily allowance for Zinc in human males and females aged 31 to 50 years is 11 and 8 mg/day respectively (IOM, 2001). Zinc is necessary for blood clotting, proper growth, thyroid function, and DNA and protein synthesis. It plays a crucial role in the storage and secretion of insulin, which increases glucose uptake (Brender, 2010; Maret, 2013). Zinc intake beyond the permissible limits, however, exerts toxic effects on blood lipoprotein levels, the immune system and copper levels (Fosmire, 1990).

Manganese (5.41 ± 0.06 mg/100g in the extract) plays a significant structural role in chloroplast membrane systems and has been adduced to be responsible for taste, color and smell. It is a cofactor for DNA, RNA and fatty acid synthetic enzymes (Imelouane *et al.,* 2011). Deficiency in Mn causes bleeding disorders, while excess Mn can lead to leg cramps, speech disorders and encephalitis (EFSA, 2013). The concentration of Cu in the ethyl acetate extract of *O. basilicum* was found to be 1.25 ± 0.12 mg/100g. Copper is an essential constituent of redox enzymes (IOM, 2001). WHO and FAO have not set the regulatory limits for Copper in herbal medicines, though China and Singapore set the limits for Copper in herbal medicines at 20 and 150 mg/kg respectively (Ullah *et al.,* 2012). The recommended dietary allowance for Copper is 900 µg/d for people aged 31-50 years (IOM, 2001). Deficiency of Cu leads to cardiovascular diseases, bone marrow

disorders, anemia and problems with the nervous system (Mlitan *et al.,* 2014). Copper is also a component of many enzymes including cytochrome oxidase, ceruloplasmin and lysyl oxidase (Indrayan *et al.,* 2005). However, excess intake of Cu can cause abdominal pain, dermatitis, diarrhea, irritation of the upper respiratory tract, nausea and liver damage (Martin & Griswold, 2009; Ullah *et al.,* 2012).

In this study, Phosphorous was found at a concentration of 7.33 ± 0.08 mg/100g in the *O. basilicum* extract. It is an important element for maintaining blood-sugar levels and normal heart contractions. It's also essential in bone growth, cell growth, kidney functions and in the maintenance of the body's acid-base balance. Cells use phosphate in transporting cellular energy in the form of ATP and other high energy phosphate compounds (IOM, 1997; Nile and Khobragade, 2009). The storage form of phosphorous in the body is phytic acid, which is an anti-nutrient due to its high affinity for Zinc, Calcium and Iron (Abebe *et al.,* 2007). Lithium (6.73 ± 0.09 mg/100g) and cobalt (0.08 ±0.02 mg/100g) were also detected in the ethyl acetate leaf extract of *O. basilicum* but Selenium and Chromium were not detected.

Heavy metal determination in biological, environmental and food samples is of importance due to their toxic effects. Lead and Cadmium accumulate in biological systems, thereby becoming contaminants along the alimentary canal. Heavy metals have harmful effects in biological systems, affecting organ systems such as the gastrointestinal, nervous, skeletal, reproductive and biochemical activities (Guil *et al.,* 1998). Cadmium, Hg, As and Pb were not detected in the extract of *O. basilicum*. The

below detectable limits of these heavy metals could be attributed to the lack of industrial activities where the plant was collected and its location that was far away from roads with heavy traffic. Nabulo *et al.* (2005), documented that the concentration of some heavy metals in plants decreased with increasing distance from roads that are associated with heavy traffic. The WHO and FAO set the acceptable limit for cadmium and lead in medicinal herbs at 0.3 mg/kg and 10 mg/kg respectively (WHO 2005; WHO 2006).

These results show that *O. basilicum* contains a considerable amount of essential and trace mineral elements and can therefore be used to supplement the daily allowance needed by the body. The fact that heavy metals were not detected in the extract could be taken as one of the markers of the safety of *O. basilicum*.

5.1.3 Antibacterial Activity of the Ethyl Acetate Leaf Extract of *O. basilicum*

The emergence of multidrug resistant strains of pathogens such as *S. aureus, E. coli* and *P. aeruginosa* has increased research in medicinal plants over the last few years. Some of the antibiotics upon which resistance has been documented include macrolides, penicillins, tetracyclines, lincosamides and gentamycin (Mehreen *et al.,* 2016). In this study, *O. basilicum* was recommended for antibacterial evaluation as it is used by herbal practitioners for the treatment of bacterial diseases in Mbeere.

The ethyl acetate extract of *O. basilicum* inhibited the growth of all the bacterial strains used in this study. The scale used for determining the strength of antibacterial activities was 8-13 mm: low inhibition; 14-19 mm: moderate inhibition; ≥ 20 mm: high inhibition (Ambrosio *et al.,* 2017). The results indicated that the extract contained a variety of

bioactive compounds that dissolved in ethyl acetate. These bioactive compounds are responsible for the antibacteial activities exhibited (Omojate *et al.,* 2014).

The antibacterial effects of *O. basilicum* on the bacterial strains ranged from 27.00 ± 2.00 mm on the quality control strain of *P. aeruginosa* to 17.33 ± 0.58 mm on the isolate of *S. aureus*. Mishra and Mishra (2011), Prasad (2012), Alo *et al.* (2012), Khalil (2013) and Odebisi-omokanye *et al.* (2016) also reported good inhibitory effects of Ocimum spp on both Gram negative and Gram positive bacteria. The extract was most effective on *P. aeruginosa*. Even though a broad spectrum antibacterial activity was exhibited, the extract showed better efficacy on the Gram positive bacteria compared to the Gram negative bacteria. The difference in susceptibility indices could be attributed to the fact that Gram negative bacteria have a stable peptidoglycan layer that allows the bioactive compounds into the cytoplasm at a slower rate compared to the Gram positive bacteria. This outcome is replicated by the study done by Yamani *et al.* (2016) but differs from the study done by Mahmood *et al.* (2008) and Rathnayaka (2013) who documented that a species of Ocimum (*O. sanctum*) exhibited better inhibitory effects on Gram negative bacteria compared to Gram positive bacteria.

The inhibitory effects of *O. basilicum* extract was dose dependent, decreasing with subsequent decrease in extract concentration. Oboh (2010) documented that the antibacterial activity of ethanolic extract of *O. gratissimum* on *S. aureus* and *P. aeruginosa* increased with respect to an increase in the concentration of the extract and vice versa.

On MRSA and *S. aureus*, Gentamycin exhibited the highest inhibitory potential followed by the highest concentration of the extract. There was no resistance from all strains of *S. aureus* on Gentamycin. However, the isolate of *S. aureus* exhibited resistance to Neomycin. There was a significant difference in the zones of inhibition between the plant extract and Gentamycin. The 0.25 gm/ml extract concentration exhibited a zone of inhibition significantly lower than those of other extract concentrations. This could be attributed to a lower concentration of bioactive compounds. The effect of Gentamycin on *S. aureus* growth was statistically significant between the clinical isolate and the standard clinical strain. This could be attributed to the fact that the clinical isolate was acquiring resistance towards Gentamycin. This difference was not observed on the extract indicating its potency in inhibiting bacterial growth. Khalil (2013) while studying the antibacterial effects of *O. basilicum* reported this herb has antibacterial effects.

On *E. coli* and *P. aeruginosa*, Gentamycin had the highest zone of inhibition on both standard strains and the clinical isolates. However, the significant difference in the activity of gentamycin between the isolate and clinical standard of *E. coli* could be attributed to the onset of resistance. The isolates of both *P. aeruginosa* and *E. coli* exhibited resistance to neomycin. The slight reduction in the activity of *O. basilicum* on the clinical isolate was not significant compared to the activity on the standard strain. This could be attributed to the synergistic effects of the phytochemicals present in the ethyl acetate extract of *O. basilicum*. These results have been replicated in other species of Ocimum (Mahamood *et al.,* 2006, Eswar *et al.,* 2016; Yamani *et al.,* 2016).

Ocimum basilicum extract, using the micro-well dilution method, exhibited an MIC of 62.5 mg/ml on the strains of *S. aureus* and *E. coli* while the MIC on *P. aeruginosa* was 125 mg/ml. This result is corroborated by Caamal-Herrera (2018), who documented MIC at 250 µg/ml while using the ethanolic extract of *O. basilicum*. These figures were, however, high from those found in a study done by Odebisi-Omokanye (2016), who found low MIC values in a study using *O. gratissimum*. Alo *et al.* (2012), recorded MIC values at 500 mg/ml using extracts of *O. gratissimum*.

The antibacterial effects could be due to the synergistic effects of the active compounds in the ethyl acetate extract of *O. basilicum*. In this study, GC-MS analysis identified different phytochemicals with associated antibacterial activities. Nootkatone is a phytochemical that has been shown to have antibacterial effects on *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Corynebacterium diphtheriae* and *Bacillus cereus* (Yamaguchi, 2019). It has been postulated that nootkatone exhibits an antibacterial effect by targeting metabolites or structures that are specific to Gram-positive bacteria such as the peptidoglycan component of cell walls. It has been shown that synthetic retinoids contain an isoprene unit that is capable of killing MRSA by penetrating and disrupting the lipid bilayers (Kim *et al.,* 2018). There is a possibility that nootkatone inhibits bacterial growth by acting on the synthetic pathway of peptidoglycan.

Dahham *et al.* (2015) and Schmidt *et al.* (2006) documented that *β*-caryophyllene had antibacterial effects and pronounced antifungal effects (Schmidt *et al.,* 2006; Dahham *et al.,* 2015). Triterpenes such as lupeol acetate found in this extract have also been shown

to have antibacterial activities (Copp & Pearce, 2007; Fontanay *et al.,* 2008; Awolola *et al.,* 2014; Barbieri *et al.,* 2017). The sesquiterpene Zierone, in a synergistic effect with α-gurjunene, was also shown to have antibacterial effects on *Bacillus subtilis* and *E. coli* (George *et al.,* 2015). The methyl ester, 11-Eicosenoic acid, has been shown to have antibacterial activities on several pathogens including *S. aureus, E. coli, Salmonella typhi,* Enterobacter and Cryptococcus (Suresh *et al.,* 2014). Indeed, it has been shown elsewhere that the methyl esters of fatty acids exhibit antibacterial and antifungal activities (Lima *et al.,* 2011; Canales *et al.,* 2011). The exact mechanisms by which fatty acids impose their antibacterial effects remain unknown. It has, however, been hypothesized that these molecules induce peroxidative processes that inhibit bacterial fatty acid synthesis. Fatty acids may also interact with cellular membranes, thereby causing leakage of molecules from the cells, reduction of nutrient uptake or inhibiting cellular respiration (Zheng *et al.,* 2005). Bicyclic amines have also been shown to have antibacterial effects (Roig-Molina *et al.,* 2018).

The observed antibacterial activity in this study could also have been due to the presence of tetradecanaal and tridecanol. These are long chain alcohols, which exhibit their antibacterial activity by damaging cellular membranes, thereby, leading to leakage of K+ ions together with subsequent reactions that lead to further leakage (Togashi *et al.,* 2007). Compounds that possess an alkyl chain have attracted attention due to the novel attributes they possess (Kubo *et al.,* 2002; Kajiya *et al.,* 2004; Stapleton *et al.,* 2004; Shibata *et al.,*

2005). They are capable of promoting antibacterial activity and resensitizing methicillin susceptible and resistant *S. aureus* to antibiotics (Togashi *et al.,* 2007).

Methylated flavonoids such as 6,3'-Dimethoxyflavone have been found to exhibit antimicrobial activities. A derivative of dimethoxyflavone has been established to have a broad spectrum antibacterial activity. These derivatives were found to be potent against *E. coli, P. aeruginosa* and *S. aureus* (Teffo *et al.,* 2010; Omosa *et al.,* 2014). Benzo[*h*]quinolones have been used to obtain novel compounds with antibacterial activities on *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes* (Naik *et al.,* 2007). 1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl- has also been shown to exhibit a broad spectrum antibacterial activity (Sun *et al.,* 2018).

In conclusion, the ethyl acetate leaf extract of *O. basilicum* exhibited antibacterial effects on MRSA, *S. aureus, P. aeruginosa* and *E. coli*. These antibacterial effects are attributed to its phytochemical composition. Therefore, this study supports the traditional use of *O. basilicum* in the treatment bacterial diseases. It also provides an important basis for the use of ethyl acetate extract of *O. basilicum* to control infectious diseases

5.1.4 Immune Modulation Effects of Ethyl Acetate Leaf Extract of *O. basilicum*

Different herbs in the family Ocimum including *O. sanctum*, *O. grattissimum* and *O. basilicum* have been reported to be immune modulators (Logambal *et al.,* 2000; Mediratta *et al.,* 2002; Pattanayak *et al.,* 2010; Tsai *et al.,* 2010; Bhat *et al.,* 2015). This

study offers fascinating evidence of *in vivo* immunomodulatory effects of the ethyl acetate leaf extract of *O. basilicum.*

Myelosuppression is a decrease in blood cell production. Cyclophosphamide is a potent immunosuppressive and cytotoxic agent that acts at different levels on immune cells by inhibiting both humoral and cell mediated immunity. It also decreases hemoglobin, red blood cell and white blood cell counts through bone marrow suppression (Patra *et al.,* 2012). Metabolism of cyclophosphamide releases active alkylating agents such as aldophosphamide, 4-hydroxycyclophosphamide and acrolein that interfere with DNA synthesis in rapidly dividing cells such as stem cells, thereby leading to cell death. Cyclophosphamide also distorts the DNA of tissues with a high cellular turnover such as the bone marrow (Patra *et al.,* 2012).

Levamisole hydrochloride has been shown to boost immunity in infectious diseases, leprosy and cancer in humans (Kar *et al*., 1986; Mutch *et al*., 1991; Katoch, 1996; Szeto *et al*., 2000). Symoens and Rosenthal (1977) documented that levamisole enhances the immune system by restoring T-lymphocyte and phagocyte functions in immune deficient hosts, but does not improve immune responses above normal levels in immunologically competent hosts. The drug's actions are moderated by the interactions between the T-cell recruiting ability of the sulphur moiety and the cholinergic effects of the imidazole ring. It also enhances the protective effects of some vaccines and is advantageous in some chronic infections, immune-deficient conditions and neoplastic diseases (Panigraphy *et al*., 1979). Treatment of dendritic cells with levamisole hydrochloride increases the presentation of CD80, CD83, CD86 and HLA-DR molecules on the cell membrane, as

well as the production of IL-10 and IL-12 p40. Levamisole hydrochloride treated human dendritic cells also enhance T cell activation towards type 1 Th immune responses by inducing interferon-γ secretion (Chen *et al.,* 2008).

Bone marrow is a major site for the sustained proliferation of vital blood cells. It is a source of a variety of important cells involved in immune responses. The high degree of cellular proliferation taking place in the marrow makes it sensitive to drugs. Thrombocytopenia and leukocytopenia result when there is loss of stem cells or when the bone marrow fails to generate new blood cells (Heroor *et al.,* 2012). In this study, injection of cyclophosphamide at 20 mg/kg body weight led to a decline in peripheral hematological parameters including RBC, WBC and Hb.

Pretreatment with *O. basilicum* extracts prevented myelosuppression in aspect of the total RBC, Hb and WBC counts. This was indicated by the increase in Hb, WBC and RBC's in the *O. basilicum* administered mice compared to the cyclophosphamide administered mice. The extract exhibited a dose dependent effect in counteracting myelosuppression induced by cyclophosphamide. Studies by Shah *et al.* (2009) and Heroor *et al.* (2012) corroborates these findings. A higher dose of the extract was shown to have a higher activity than the lower doses with dissimilar levels of significance in activity. The ability of the extracts to stimulate the hematopoietic system can be attributed to the presence of phytochemicals (Scarpa and Ninfali, 2015).

Scarpa and Ninfali (2015) reviewed that phytochemicals are capable of targeting the Wnt/ β-catenin pathway implicated in the destruction of stem cells and pathogenesis of different cancers. It has been demonstrated that the absence of Wnt signaling results in β-catenin remaining in the cytoplasm, thereby, forming a complex with glycogen synthase kinase (GSK-3β) that phosphorylates β-catenin thereby undergoing degradation. Activation of the Wnt pathway inhibits GSK-3β, thereby, blocking β-catenin phosphorylation. Unphosphorylated β-catenin is stable and translocates to the nucleus. In the nucleus it activates the transcription factors T cell factor-lymphoid enhancer factor (TCF-LEF) by binding to it which in turn increases self-renewal and proliferation of stem cells.

A haemagglutination test confirmed the effect of the ethyl acetate leaf extract of *O. basilicum* on the humoral immune system. This encompasses the interactions of B cells and antigens and their subsequent proliferation and differentiation into antibody producing cells. Antibodies perform their functions by binding to antigens and neutralizing them or facilitating their elimination by crosslinking to form latex that's readily ingested by a phagocytic cell (Sudha *et al.,* 2010). The effect of the ethyl acetate leaf extract of *O. basilicum* was tested by rabbit erythrocyte hemagglutinin antibody titer response. The extract counteracted the suppression effects of cyclophosphamide on humoral antibody responses. This implies that it improved the responsiveness of T and B lymphocyte, and macrophages in antibody synthesis.

Mice administered with cyclophosphamide exhibited a significant reduction in hemagglutinin antibody titer compared to all the other treatment groups. The extracts showed a dose dependent effect on the humoral responses with the highest activity in the 300 mg/kg body weight dose administered mice being significant from the other treatment groups. However, this effect was not statistically significant from the positive control group. These results showed that different active phytochemicals in the extract enhanced RRBC's binding to B-memory cells and, therefore, stimulating them to differentiate to antibody secreting plasma cells that enhanced secondary antibody responses. These results are in tandem with those of Dashputre and Naikwade (2010) and Vaghasuya *et al.* (2010) who used *O. basilicum* and *O. sanctum* in their studies respectively. Studies done using different plants have also exhibited the same effects on humoral responses (Sudha *et al,* 2010; Al Sheyab, 2012; Sudan *et al.,* 2014; Une and Doshi, 2016).

The percentage neutrophil counts in blood samples of mice administered with the extract was evaluated. There was a significant difference in % neutrophil counts in groups administered with the extract compared to the groups administered with cyclophosphamide and DMSO. However, the percentage neutrophil counts in mice administered with 300 mg/kg body weight was comparable to the mice administered with levamisole hyrochloride and significantly different from the other groups administered with *O. basilicum* extract. The low dose of 100 mg/kg body weight was found to be more effective than the high dose of 200 mg/kg body weight. This effect was also observed by Sudha *et al.,* (2010), in their study using *Moringa oleifera*.

Secretion of cytokines by activated immune cells is necessary for the margination and extravasation of phagocytes, mainly neutrophils. The increase in neutrophil index due to enhanced neutrophil adhesion to nylon fibers indicate that *O. basilicum* maybe useful in promoting phagocytosis. The increase in neutrophil adhesion induced by *O. basilicum* extracts correlates with the process of cell margination within the blood vessles (Dashputre and Naikwade, 2010). These results are in tandem with those of Jeba and Rameshkumar, (2013) who demonstrated that *O. sanctum* had a significant effect on neutrophil adhesion while *O. basilicum* did not. In a different study, Dashputre and Naikwade (2010), demonstrated that *O. basilicum* had significant effects on neutrophil adhesion.

Delayed type hypersensitivity is part of the processes in tumor immunity, graft rejection and immunity to intracellular pathogens such as tuberculosis (Elgert, 1996). The maximum reaction time occurs between 48 to 72 hours and this process is mediated by T cells that cause inflammatory reactions to auntoantigens or exogenous antigens. Response to exogenous antigens involve antigen-presenting cells (APC) and T cells that secrete cytokines which stimulate a local inflammatory reaction in a sensitized individual. Antigen-presenting cells bind and present antigens to antigen-specific T cells that become sensitized. Cytokines secreted by APCs, keratinocytes, and T cells recruit antigen non-specific T cells and macrophages to participate in a local inflammatory response (Barailler *et al.,* 2019).

The ethyl acetate extract of *O. basilicum* showed there was a significant increase in footpad edema at the 6th and the 8th hour in mice administered with the extract compared to cyclophosphamide and the negative control. However, at the 48th hour, the footpad edema was less in the group administered with the extract compared to the negative control but the difference was not statistically significant. These results are, however, not in tandem with those of Sahu *et al.* (2013).

Delayed type hypersensitive reactions induced by RRBC's were not inhibited by the ethyl acetate extract of *O. basilicum*. The extract therefore did not inhibit T lymphocytes required for the expression of DTHR (Vinothapooshan and Sundar, 2011). This reaction is dependent on memory T cells that produce interferon gamma producing CD4+ or CD8+ T cells (Furr, 1998; Black, 1999; Biedermann *et al.,* 2001). The reference drug, levamisole hydrochloride, elevates cGMP in lymphocytes. Its imidazole ring is among the active moieties involved in the functional increase of peripheral macrophages and T-cells, therefore, levamisole hydrochloride does not inhibit DTHR (Alan *et al.,* 2001). These results differed from those obtained in different studies that used dissimilar herbal plants (Sahu *et al.,* 2010; Tilwari *et al.,* 2011; Onwuka *et al.,* 2016).

Delayed type hypersensitive reactions encompass two phases; a sensitization phase after contact with an antigen in which Th1 cells are activated and clonally expand with the aid of antigen presenting cells with a class II major histocompatibility complex molecule. Subsequent antigenic exposure induces the effector phase of DTH responses. In this phase, cytokines that recruit and activate non-specific inflammatory mediators and

macrophages are secreted by Th1 cells (Vinegar *et al.,* 1987; Bafna *et al.,* 2004). Delay in the onset of DTH reflects the time cytokines take to induce macrophage recruitment and activation (Bafna *et al.,* 2004).

Hematological parameters in DTH animal models were elevated in mice administered with the extract in a dose dependent manner suggesting that the *O. basilicum* leaf extract was capable of potentiating the effect of cyclophosphamide on the stem cells.

Phagocytosis is important in removal of foreign bodies, microorganisms, malignant cells, inorganic particles, tissue debris, dead or injured cells from the body. The concept of carbon clearance is correlated with enhanced phagocytic activity (Miller and Peacock, 1991). *Ocimum basilicum* exhibited a dose dependent increase in the rate of carbon clearance and phagocytic index. However, the rate of carbon clearance and the phagocytic index of the extract was not statistically significant from that of levamisole hydrochloride or from the group administered with the DMSO.

The dose dependent increase in the rate of carbon clearance and phagocytic index indicates that the phagocytic functions of nonspecific immunity and mononuclear macrophages is enhanced by the extract of *O. basilicum*. Macrophage phagocytosis is vital against smaller parasites and is enhanced by opsonisation of parasites by the complement system and antibodies thereby resulting to enhanced parasite clearance from the body (Pallabi *et al.,* 1998). The extract enhanced phagocytic functions by demonstrating a clearance rate of carbon particles by the immune cells of the

reticuloendothelial system. These results are in tandem with those documented by Dashputre and Naikwade (2010) in their study using *O. basilicum*. Doshi and Une (2015) and Sudha *et al.* (2010) observed the same results when using different herbal plants.

In conclusion, *Ocimum basilicum* has immune modulation properties on both nonspecific and specific immune mechanisms. These properties could be attributed to the presence of phytochemicals as well as mineral elements acting as co-factors. This traditional medicinal plant, therefore, holds promise for use as an immune stimulating agent and its use is therefore validated.

5.1.5 *In vivo* Safety Profile of the Ethyl Acetate Leaf Extract of *O. basilicum*

Traditional alternative and complementary medicine has been in use since the days before the dawn of civilization. Its popularity worldwide has challenged orthodox practices in several ways (Catarino *et al,* 2016). However, there is a lack of regulation in the use of traditional medicine that has had a negative impact. This void in regulation could be due to a paucity of data and adequate research methods for evaluating herbal medicinal products (Ekor, 2014; Catarino *et al.,* 2016). Several traditional medicines have been characterized with toxicity (De Smet, 2004; Awodele *et al.,* 2013; Skalicka *et al.,* 2017). The Food and drug administration has warned on the potential toxic effects of medicinal plants (De Smet, 2004). Toxicological testing is important to popularize acceptance and standardize the market of herbal medicines (Kale *et al.,* 2016). It is also important in aspects of drug development and for the extension of their therapeutic use as well as help pinpoint information on the toxic reactions that are relevant to the substance under

evaluation (De Smet, 2004; Jordan *et al.,* 2010; Ekor, 2014; Akindele *et al.,* 2014; Van andel *et al.,* 2015; Kale *et al.,* 2016; Catarino *et al.,* 2016; Skalicka *et al.,* 2017; Awodele *et al.,* 2018; Kale *et al.,* 2018; Ching *et al.,* 2018; Kale *et al.,* 2019). Safety evaluation of herbal medicines is of paramount importance, given the high number of individuals consuming them with the purpose of curbing infections. Decision makers and researchers should be able to obtain a variety of evidentiary sources about safety of these herbal medicines (Diallo *et al.,* 2014; Akindele *et al.,* 2015; Kale *et al.,* 2018; Kale *et al.,* 2018).

Safety evaluation of the ethyl acetate leaf extract of *O. basilicum* showed that intragastric administration of doses up to 1000 mg/kg body weight did not result into demonstrable toxic effects or death in mice. There were no observable changes in mice behavior or demeanor during the dosing period. These results are in tandem with those of Uma *et al.* (2013) and Sadavish (2010), who found no observable changes in rats administered with *Ocimum tenuiflorum (O. tenuiflorum)* extracts and mice administered with extracts of *O. sanctum* in safety studies.

Rasekh *et al.* (2012), recorded no observable changes in mice treated with a hydro-alcoholic extract of *O. sanctum*. However, they do not tally with those of Nunez *et al.* (2017) who observed slight edemas in mice treated with *O. sanctum* from Cuba. Kuete (2014), in their safety survey of African medicinal plants, *O. gratisimum* was found to have toxic effects on the behavior of animals. The differences in the effects observed could be attributed to the different regions from which the plants were collected, the different species and solvents used as well as different concentrations used in the studies.

Weight modulation is a very important characteristic of numerous medicinal agents. Body weight variations indicate physiological alterations in liver functioning, hormonal activities, reduced protein metabolism etc. (Diallo *et al.,* 2014; Akindele *et al.,* 2015; Kale *et al.,* 2018; Kale *et al.,* 2018). Body weight is a vital aspect when monitoring the health of an organism. A decrease in body weight is the first indicator for the onset of adverse reactions. A toxic dose is that which causes a 10% decrease in body weight and is considered the one which produces minimal toxic effects irrespective of whether it is accompanied by other changes (Sadashiv, 2010). Administration of Swiss albino mice with the leaf extract of *O. basilicum* did not result in statistically significant differences in body weights or organ weights between the treatment groups and the control group. There was a gradual weekly increase in body weights of the mice from the start of dosing to the 28th day suggesting that the leaf extract of *O. basilicum* did not contain metabolites that interfered with feed intake, metabolism or enhanced proteolysis of skeletal muscles thereby retarding growth. Phytochemicals affect weight via appetite suppression, energy expenditure and through blocking fat-glucose absorption (Pasman *et al.,* 2008; Tucci *et al.,* 2010). These results are in tandem with those found by Rasekh *et al.* (2012), Uma *et al.* (2013), Gautam and Goel, (2014) and Nunez *et al.* (2017) in their studies using different species of Ocimum.

Organ weights and relative organ weights to body weights of mice did not exhibit significant differences between the treated groups and the control group even with a high dose of 1000 mg/kg body weight administered for 28 days. There was observed a dose dependent decrease in the weight of lungs across the groups, however, this decrease was

not significant. The extract at the administered doses, therefore, did not have toxic effects on individual organs. These results are corroborated by Rasekh *et al.* (2012) and Gautam and Goel, (2014) in their studies.

The bone marrow/hematopoietic stem system is regarded as an important index of pathological and physiological status in animals as it is a sensitive target for toxic compounds. The analysis of hematological parameters is paramount for risk evaluation because changes in the blood has a high predictive value for human toxicity when data is translated from animal studies (Olson *et al.,* 2000; Mukinda *et al.,* 2007). Exposure of mice to the ethyl acetate leaf extract of *O. basilicum* exhibited a dose dependent effect on red blood cell indices, with the exception of Mean Corpuscular Hemoglobin Concentration. However, the increases were small and transient as they were not significantly different from the control. Red blood cells, Hb and MCV are linked to the total population of red cells; MCH and MCHC mathematically define hemoglobin concentration and suggest the maintenance of the oxygen carrying capacity of blood (Mahmoud, 2013). From the findings, it is evident that the extract did not interfere with blood flow or oxygen carriage and circulation by the red blood cells. These findings are in agreement with those of Gautam and Goel (2014) and Sharwan *et al.* (2016). They, however, disagree with those of Rasekh *et al.* (2012) who found significant decreases in RBC, Hb and HCT concentrations in their study of the safety of *O. sanctum* in rats.

White blood cells, including granulocytes, monocytes and lymphocytes exhibit either quantitative or qualitative disorders in cases of toxicity. Quantitative alterations have normal appearing cells circulating in abnormal quantities (Blumenreich, 1990). In this

study, quantitative analysis of white blood cell indices indicated that the ethyl acetate leaf extract of *O. basilicum* did not exhibit toxic effects. Normally, monocytes and lymphocytes are elevated in cases of chronic infections while granulocytes are elevated in cases where there is decreased egress in circulation, increased production and release from storage compartments or demargination (Blumenreich, 1990). Results from Rasekh *et al.* (2012), Gautam and Goel (2014); Sharwan *et al.* (2016) are in agreement.

There was significant thrombocytopenia in the group that received the highest dose of ethyl acetate leaf extract of *O. basilicum*. Therefore, it would seem that the extract had compounds that caused the immune system to destroy platelets, or the extract had compounds that destroyed platelet progenitors. Platelets are important in homeostasis. Mean platelet volume is an indicator of platelet functions such as platelet aggregation, release of platelet factor 4, release of thromboxane A_2 and the expression of glycogen 1b receptors (Ofem *et al.,* 2012). This study showed that MPV decreased in a dose dependent manner though the decrease was not significant. Mean platelet volume is used as an indicator of atherothrombosis. Elevations in MPV have been documented in persons with diabetes, stroke and metabolic syndromes (O'Malley, 1995; Tavil *et al.,* 2007). There was no significant effect on plateletcrit and platelet distribution width.

The liver is a vital organ in metabolism. It's a site of cholesterol synthesis, disposal and degradation as well as the site for generation of free glucose from glycogen stores (Anderson and Borlak, 2008). As no significant changes were observed in cholesterol levels, it suggests that the extract did not have any effect on carbohydrate and lipid

metabolism in mice. In addition, hepatic toxicity affects transaminases which are good biomarkers for toxicity and liver functions (Rahman, 2001; Hilaly *et al.,* 2004).

Transaminases tend to rise when there is damage to parenchymal liver cells. A three fold rise in transaminases is usually a sign of toxicity in the liver (Anderson and Borlak, 2008). In this study, ALT, AST as well as ALP did not exhibit treatment related elevations even at doses of 1000 mg/kg body weight compared to the control group. Additionally, serum AST is of both cytoplasmic and mitochondrial origin and any elevation can be taken as a sign of cell damage leading to the overflow of enzymes into the serum (Mukinda and Eagles, 2010). Therefore, there being no significant increases in ALT and AST suggests that the administration of the ethyl acetate leaf extract of *O. basilicum* did not have a net negative effect on hepatocytes and metabolism in mice.

Serum albumin and bilirubin concentrations indicate synthetic and secretory liver functioning and are important in ascertaining the different types of liver damage (Oboh and Rocha, 2007). Bilirubin is a major by-product for the breakdown of old red blood cells. The fact that it is removed from blood by the liver makes it a good indicator for hepatic functions. Bilirubin elevation in blood occurs due to decreased liver uptake (due to liver disease or hepatic toxicity) or because of increased bilirubin production (Egesie *et al.,* 2006). The results in this study indicate that there was no significant difference in total bilirubin and conjugated bilirubin levels in mice administered with the ethyl acetate leaf extracts of *O. basilicum* compared to the control. This observation also suggests that the extract was not toxic to the liver. This finding is in tandem with that of Ojo *et al.* (2013), in their study using *O. gratissimum.*

Albumin is a major protein circulating in the blood stream and provides a reliable test in assessing the degree of liver damage (Yakubu *et al.,* 2003). Low serum levels are associated with low protein intake. There was no significant difference in albumin as well as in total protein levels between mice administered with the extract and the control mice.

The ethyl acetate extract had compounds with gastroprotective, hepatoprotective, antihyperglycemic and hypolipidemic effects. β- amyrin brings about gastroprotection through the activation of primary afferent neurons sensitive to capsaicin. It also aids in hepatoprotection by attenuating the rise in serum AST and ALT activities. It has also been documented to increase hepatic glutathione and reduce histopathological changes. It has also been shown to significantly decrease total cholesterol, glycemic and serum tyglyceride levels in diabetic animals. Lupeol acetate has been shown to be hepatoprotective (Sudhahar *et al.,* 2008).

The kidneys are a major site of damage as a result of toxicity. This is evidenced in standard clinical care and drug development. Nephrotoxicity due to drug toxicity has been established to contribute to 19-25% of all cases of kidney injury. Given the societal cost of nephrotoxicity, it is vital that preclinical studies on herbal medicine toxicity are done in drug development. Serum creatinine and blood urea nitrogen are the commonly used biomarkers in detection of nephrotic damage (Bonventre *et al.,* 2010). An elevated renal biomarker is an indication that poor excretion may occur (Shimoishi *et al,* 2007). Results in this study indicate that serum urea and creatinine levels were not significantly different between the groups administered with the extract and the control. Therefore, the extracts did not confer nephrotic damage to the mice. Rasekh *et al.* (2012); Gautam and

Goel (2014) in their studies recorded equal results using different herbal plants. However, when using *O. gratissimum*, Ogundipe *et al*. (2016) recorded elevated levels of creatinine and urea in their safety study while using the same plant, Anigbogu and Uzoaga (2006) reported that it reduced renal functions, though not significantly.

These results suggest that the oral LD50 of this extract is higher than 1000 mg/kg body weight. Therefore, the ethyl acetate leaf extract of *O. basilicum* can be categorized as being highly safe because substances with their LD50 higher than 50 mg/kg body weight are non-toxic (Ramadan *et al.,* 2010). The results obtained from this study support the notion that the plant is safe for use.

5.2 Conclusions

i. The ethyl acetate leaf extract of *O. basilicum* contained bioactive phytochemicals and nutritionally important mineral elements while heavy metals were not detected.
ii. The ethyl acetate leaf extract of *O. basilicum* exhibited antibacterial activities on both gram positive (*S. aureus*, MRSA) and gram negative (*E. coli* and *P. aeruginosa*) bacteria.
iii. The ethyl acetate leaf extract of *O. basilicum* was capable of modulating the immune system in Swiss albino mice.
iv. Tested for its *in vivo* safety, the extract did not induce pharmacotoxicological effects in Swiss albino male mice.

5.3 Recommendations

i. *Ocimum basilicum* extract can be used as a nutiritional supplement for the body's mineral element requirements.

ii. *Ocimum basilicum* can be used to treat infectious diseases caused by both gram negative and gram positive bacteria.

iii. In cases where the immune system needs to be boosted, *O. basilicum* has the ability to stimulate the immune system.

iv. The *in vivo* safety of the extract means that it can be used to treat infections or as a food supplement without any toxic effects.

5.4 Suggestions for Further Studies

i. Different solvents should be used in extraction and the extracts used on a variety of drug resistant strains of pathogenic bacteria.

ii. Fractionation of the bioactive phytochemicals should be done and their individual capabilities in immune stimulation and antibacterial activities determined.

iii. Research on the effect of *O. basilicum* on the molecular markers for toxicity and histopathology.

iv. The efficacy of *O. basilicum* extracts on other pathogens such as parasites and viruses should be evaluated.

REFERENCES

Abd-Elmonsef, M. M., Elsharawy, D., & Abd-Elsalam, A. S. (2017). Mechanical ventilator as a major cause of infection and drug resistance in intensive care unit. *Environmental Science and Pollution Research*, 1-6 https://doi.org/10.1007/s11356-017-8613-5

Abebe, Y., Bogale, A., Hambidge, K. M., Stoecker, B. J., Bailey, K., & Gibson, R. S. (2007). Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. *Journal of Food Composition and Analysis*, *20*(3-4), 161-168.

ad Khalil, A. (2013). Antimicrobial activity of ethanolic extracts of *Ocimum basilicum* leaf from Saudi Arabia. *Biotechnology*, *12*(1), 61-64.

Adhikari, R. P., Ajao, A. O., Aman, M. J., Karauzum, H., Sarwar, J., Lydecker, A. D., & Roghmann, M. C. (2012). Lower antibody levels to *Staphylococcus aureus* exotoxins are associated with sepsis in hospitalized adults with invasive S. *aureus* infections. *The Journal of Infectious Diseases*, *206*(6), 915-923.

Agarwal, K., & Varma, R. (2014). Studies on antioxidant activity and phytochemical screening of selected medicinal plant *Ocimum gratissimum* l. *International Journal of Pharmaceutical Sciences and Research, 5*(8), 3520.

Aiken, A. M., Mutuku, I. M., Sabat, A. J., Akkerboom, V., Mwangi, J., Scott, J. A. G., & Grundmann, H. (2014). Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. *Antimicrobial Resistance and Infection Control*, *3*(1), 1.

Akindele, A. J., Adeneye, A. A., Salau, O. S., Sofidiya, M. O., & Benebo, A. S. (2014). Dose and time-dependent sub-chronic toxicity study of hydroethanolic leaf extract of *Flabellaria paniculata* Cav.(Malpighiaceae) in rodents. *Frontiers in Pharmacology*, *5*, 78.

Akindele, A. J., Unachukwu, E. G., & Osiagwu, D. D. (2015). 90 Days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Desr.) Roem. and Schult.(Convolvulaceae) in rats. *Journal of Ethnopharmacology*, *174*, 582-594.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essays*, *2*(5), 163-166.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, *48*(4), 487-491.

Al Abbasy, D. W., Pathare, N., Al-Sabahi, J. N., & Khan, S. A. (2015). Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). *Asian Pacific Journal of Tropical Disease*, *5*(8), 645-649.

Al Sheyab, F. M., Abuharfeil, N., Salloum, L., Hani, R. B., & Awad, D. S. (2012). The effect of Rosemary (*Rosmarinus officinalis*. L) plant extracts on the immune response and lipid profile in mice. *Journal of Biology and Life Science*, *3*(1).

Alam, M. T., Petit III, R. A., Crispell, E. K., Thornton, T. A., Conneely, K. N., Jiang, Y., & Read, T. D. (2014). Dissecting vancomycin-intermediate resistance in *Staphylococcus aureus* using genome-wide association. *Genome Biology and Evolution*, *6*(5), 1174-1185.

Alan, M. K., & Terry, B. S. (2001). Immunomodulators: immunosuppressive agents, tolerogens and immunostimulants. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, *10*.

Al-Bayati, F. A. (2009). Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. *Annals of Clinical Microbiology and Antimicrobials*, *8*(1), 20.

Alberti, C., Brun-Buisson, C., Burchardi, H., Martin, C., Goodman, S., Artigas, A., ... & Lepage, E. (2002). Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. *Intensive Care Medicine*, *28*(2), 108-121.

Al-Hamad, A., & Maxwell, S. (2008). How clean is clean? Proposed methods for hospital cleaning assessment. *Journal of Hospital Infection*, *70*(4), 328-334.

Ali, A. M., Rafi, S., & Qureshi, A. (2004). Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi. *Proteus*, *2*(11), 25.

Allegranzi, B., Nejad, S. B., Combescure, C., Graafmans, W., Attar, H., Donaldson, L., & Pittet, D. (2011). Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *The Lancet*, *377*(9761), 228-241.

Allen, J. E., & Sutherland, T. E. (2014, August). Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin. In *Seminars in Immunology* (Vol. 26, No. 4, pp. 329-340). Academic Press.

Alo, M. N., Anyim, C., Igwe, J. C., Elom, M., & Uchenna, D. S. (2012). Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta*. *Advances in Applied Science Research, 3*(2), 844-848.

Alqahtani, A., Hamid, K., Kam, A., Wong, K. H., Abdelhak, Z., Razmovski-Naumovski, V., & Li, G. Q. (2013). The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. *Current Medicinal Chemistry*, *20*(7), 908-931.

Ambrosio, C. M., de Alencar, S. M., de Sousa, R. L., Moreno, A. M., & Da Gloria, E. M. (2017). Antimicrobial activity of several essential oils on pathogenic and beneficial bacteria. *Industrial Crops and Products*, *97*, 128-136.

Amri, I., Hanana, M., Jamoussi, B., & Hamrouni, L. (2017). Essential oils of *Pinus nigra* JF Arnold subsp. laricio Maire: Chemical composition and study of their herbicidal potential. *Arabian Journal of Chemistry*, *10*, S3877-S3882.

Anand, K. B., Agrawal, P., Kumar, S., & Kapila, K. (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian Journal of Medical Microbiology*, *27*(1), 27.

Anderson, N. and Borlak, J. (2008). Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacological Reviews*, *60*(3), 311–357.

Anguzu, J. R., & Olila, D. (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *African Health Sciences*, *7*(3).

Anigbogu, C. N., & Uzoaga, K. T. (2006). The effects of *Ocimum gratissimum* leaf extract on cardiovascular and renal function in rats. *Nigerian Quarterly Journal of Hospital Medicine*, *16*(2), 60-65.

Araújo Silva, V., Pereira da Sousa, J., de Luna Freire Pessôa, H., Fernanda Ramos de Freitas, A., Douglas Melo Coutinho, H., Beuttenmuller NogueiraAlves, L., & Oliveira Lima, E. (2015). *Ocimum basilicum*: Antibacterial activity and association study with antibiotics against bacteria of clinical importance. *Pharmaceutical Biology*, 1-5.

Arias, C. A., Panesso, D., McGrath, D. M., Qin, X., Mojica, M. F., Miller, C., & Reyes, J. (2011). Genetic basis for *in vivo* daptomycin resistance in enterococci. *New England Journal of Medicine*, *365*(10), 892-900.

Arika, W. M., Nyamai, D. W., Musila, M. N., Ngugi, M. P., & Njagi, E. N. M. (2016). Hematological Markers of *In Vivo* Toxicity. *Journal of Hematology & Thromboembolic Diseases*. 4:236.

Arika, W. M., Ogola, P. E., Abdirahman, Y. A., Mawia, A. M., & Wambua, F., K. (2016). *In Vivo* Safety of Aqueous Leaf Extract of *Lippia javanica* in Mice Models. *Biochemistry & Physiology*. 5: 191.

Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., & Asaolu, M. F. (2012). Proximate and mineral composition of Nigerian leafy vegetables. *Journal of Food Research*, *1*(3), 214.

Aslinia, F., Mazza, J. J., & Yale, S. H. (2006). Megaloblastic anemia and other causes of macrocytosis. *Clinical Medicine & Research*, *4*(3), 236-241.

Awodele, O., Badru, W. A., Busari, A. A., Kale, O. E., Ajayi, T. B., Udeh, R. O., & Emeka, P. M. (2018). Toxicological evaluation of therapeutic and supra-therapeutic doses of Cellgevity® on reproductive function and biochemical indices in Wistar rats. *BMC Pharmacology and Toxicology*, *19*(1), 68.

Awolola, G. V., Koorbanally, N. A., Chenia, H., Shode, F. O., & Baijnath, H. (2014). Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of *Ficus sansibarica Warb*. subsp. *sansibarica* (Moraceae) extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*(3), 124-131.

Awounfack, C. F., Ateba, S. B., Zingue, S., Mouchili, O. R., & Njamen, D. (2016). Safety evaluation (acute and sub-acute studies) of the aqueous extract of the leaves of *Myrianthus arboreus P. Beauv.* (Cecropiaceae) in Wistar rats. *Journal of Ethnopharmacology*, *194*, 169-178.

Ayyanar, M., & Ignacimuthu, S. (2005). Traditional knowledge of kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. *Journal of Ethnopharmacology*, *102*(2), 246-255.

Azevedo, F., B., Barros Furieri, L., Peçanha, F. M., Wiggers, G. A., Frizera Vassallo, P., Ronacher Simões, M., & Stefanon, I. (2012). Toxic effects of mercury on the cardiovascular and central nervous systems. *BioMedical Research International*, *2012*.

Badshah, H., Ali, T., Rehman, S. U., Amin, F. U., Ullah, F., Kim, T. H., & Kim, M. O. (2016). Protective effect of lupeol against lipopolysaccharide-induced neuroinflammation via the p38/c-Jun N-terminal kinase pathway in the adult mouse brain. *Journal of Neuroimmune Pharmacology*, *11*(1), 48-60.

Bafna, A. R., & Mishra, S. H. (2004). Actividad inmunomoduladora del extracto de metanol de la cabeza floral de *Sphaeranthus indicus* Linn. *Ars Pharmaceutica*, *45*(3), 281-291.

Bajaj, Y. S. (2005). Phytochemistry, 66, 2056.

Bandeira, P. N., Pessoa, O. D. L., Trevisan, M. T. S., & Lemos, T. L. G. (2002). Metabólitos secundários de *Protium heptaphyllum* March. *Química Nova*, *25*(6B), 1078-1080.

Barailler, H., Milpied, B., Chauvel, A., Claraz, P., Taïeb, A., Seneschal, J., & Darrigade, A. S. (2019). Delayed hypersensitivity skin reaction to hydroxychloroquine: Successful short desensitization. *The Journal of Allergy and Clinical Immunology: In Practice,* 7(1), 307-308.

Barber, M. (1961). Methicillin-resistant staphylococci. *Journal of Clinical Pathology*, 14:385–93.

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sanchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research*, *196*, 44-68.

Bartley, G. E., & Scolnik, P. A. (1995). Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *The Plant Cell*, *7*(7), 1027.

Bayala, B., Bassole, I. H. N., Gnoula, C., Nebie, R., Yonli, A., Morel, L., & Simpore, J. (2014). Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. *PLoS One*, *9*(3), e92122.

Bayes, H. K., Ritchie, N. D., & Evans, T. J. (2016). Interleukin-17 Is Required for Control of Chronic Lung Infection Caused by *Pseudomonas aeruginosa*. *Infection and Immunity*, *84*(12), 3507-3516.

Becker, D. E. (2013). Antimicrobial drugs. *Anesthesia Progress*, *60*(3), 111-123.

Belbase, A., Pant, N. D., Nepal, K., Neupane, B., Baidhya, R., Baidya, R., & Lekhak, B. (2017). Antibiotic resistance and biofilm production among the strains of *Staphylococcus aureus* isolated from pus/wound swab samples in a tertiary care

hospital in Nepal. *Annals of Clinical Microbiology and Antimicrobials*, *16*(1), 15.

Belkum, A., Verkaik, N. J., de Vogel, C. P., Boelens, H. A.,Verveer, J., Nouwen, J. L., & Wertheim, H. F. (2009). Reclassification of *Staphylococcus aureus* nasal carriage types. *Journal of Infectious Diseases*, *199*(12), 1820-1826.

Bereket, W., Hemalatha, K., Getenet, B., Wondwossen, T., Solomon, A., Zeynudin, A., & Kannan, S. (2012). Update on bacterial nosocomial infections. *European Review of Medical Pharmacological Sciences*, *16*(8), 1039-44.

Berkley, J. A., Lowe, B. S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., & Maitland, K. (2005). Bacteremia among children admitted to a rural hospital in Kenya. *New England Journal of Medicine*, *352*(1), 39-47.

Bernhoft, R. A. (2013). Cadmium toxicity and treatment. *The Scientific World Journal*, *2013*, Article ID 394652, 7 pages.

Bhat, H. P., Jakribettu, R. P., Boloor, R., Fayad, R., & Baliga, M. S. (2015). Use of Ayurvedic Medicinal Plants as Immunomodulators in Geriatrics: Preclinical Studies. In *Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults* (pp. 143-149).

Bhatia, S. P., Letizia, C. S., & Api, A. M. (2008). Fragrance material review on betacaryophyllene alcohol. *Food And Chemical Toxicology;* 46(suppl 11).

Biedermann, T., Mailhammer, R., Mai, A., Sander, C., Ogilvie, A., Brombacher, F., & Röcken, M. (2001). Reversal of established delayed type hypersensitivity reactions following therapy with IL-4 or antigen-specific Th2 cells. *European Journal of Immunology*, *31*(5), 1582-1591.

Bien, J., Sokolova, O., & Bozko, P. (2012). Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *International Journal of Nephrology*, *2012*, Article ID 681473, 15 pages.

Bjarnsholt, T., Jensen, P. Ø., Fiandaca, M. J., Pedersen, J., Hansen, C. R., Andersen, C. B., & Høiby, N. (2009). *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. *Pediatric pulmonology*, *44*(6), 547-558.

Black, C. A. (1999). Delayed type hypersensitivity: Current theories with a historic perspective. *Dermatology Online Journal*, *5*(1).

Blumenreich, M. S. (1990). The white blood cell and differential count. *Clinical Methods: The History, Physical, and Laboratory Examinations [Internet]. 3rd ed. Boston: Butterworths*.

Bonventre, J. V., Vaidya, V. S., Schmouder, R., Feig, P., & Dieterle, F. (2010). Next-generation biomarkers for detecting kidney toxicity. *Nature Biotechnology*, *28*(5), 436-440.

Boocock, D. J., Faust, G. E., Patel, K. R., Schinas, A. M., Brown, V. A., Ducharme, M. P., & Steward, W. P. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiology and Prevention Biomarkers*, *16*(6), 1246-1252.

Bouvet, C., Gjoni, S., Zenelaj, B., Lipsky, B. A., Hakko, E., & Uçkay, I. (2017). *Staphylococcus aureus* soft tissue infection may increase the risk of subsequent staphylococcal soft tissue infections. *International Journal of Infectious Diseases*, *60*, 44-48.

Bradford, P. A. (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*, *14*(4), 933-951.

Brender, J. R., Hartman, K., Nanga, R. P. R., Popovych, N., de la Salud Bea, R., Vivekanandan, S., & Ramamoorthy, A. (2010). Role of zinc in human islet amyloid polypeptide aggregation. *Journal of the American Chemical Society*, *132*(26), 8973-8983.

Budak, Y. U., Polat, M., & Huysal, K. (2016). The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. *Biochemia Medica: Biochemia medica*, *26*(2), 178-193.

Caamal-Herrera, I. O., Carrillo-Cocom, L. M., Escalante-Réndiz, D. Y., Aráiz-Hernández, D., & Azamar-Barrios, J. A. (2018). Antimicrobial and antiproliferative activity of essential oil, aqueous and ethanolic extracts of *Ocimum micranthum* Willd leaves. *BMC Complementary and Alternative Medicine*, *18*(1), 55.

Canales, M., Hernández, T., Rodriguez-Monroy, M. A., Flores, C. M., Jiménez-Estrada, M., Hernández, L. B., & Ramirez, J. J. (2011). Evaluation of the antimicrobial activity of *Acalypha monostachya* Cav.(Euphorbiales: Euphorbiaceae). *African Journal of Pharmacy and Pharmacology*, *5*(5), 640-647.

Catarino, L., Havik, P. J., & Romeiras, M. M. (2016). Medicinal plants of Guinea-Bissau: Therapeutic applications, ethnic diversity and knowledge transfer. *Journal of Ethnopharmacology*, *183*, 71-94.

Chang, C. W., Beland, F. A., Hines, W. M., Fuscoe, J. C., Han, T., & Chen, J. J. (2011). Identification and categorization of liver toxicity markers induced by a related pair of drugs. *International Journal of Molecular Sciences*, *12*(7), 4609-4624.

Chau, T. A., McCully, M. L., Brintnell, W., An, G., Kasper, K. J., Vinés, E. D., & Heinrichs, D. E. (2009). Toll-like receptor 2 ligands on the staphylococcal cell wall downregulate superantigen-induced T cell activation and prevent toxic shock syndrome. *Nature Medicine, 15*(6), 641-648.

Chen, L. Y., Lin, Y. L., & Chiang, B. L. (2008). Levamisole enhances immune response by affecting the activation and maturation of human monocyte- derived dendritic cells. *Clinical & Experimental Immunology*, *151*(1), 174-181.

Chen, T. C., Da Fonseca, C. O., & Schönthal, A. H. (2015). Preclinical development and clinical use of perillyl alcohol for chemoprevention and cancer therapy. *American Journal of Cancer Research*, *5*(5), 1580.

Chen, X., Peng, L. H., Shan, Y. H., Li, N., Wei, W., Yu, L., & Gao, J. Q. (2013). Astragaloside IV-loaded nanoparticle-enriched hydrogel induces wound healing and anti-scar activity through topical delivery. *International Journal of Pharmaceutics*, *447*(1-2), 171-181.

Cheng, W., Li, J., You, T., & Hu, C. (2005). Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of *Chrysanthemum indicum* Linne. *Journal of Ethnopharmacology*, *101*(1-3), 334-337.

Chi, S. Y., Kim, T. O., Park, C. W., Yu, J. Y., Lee, B., Lee, H. S., & Kwon, Y. S. (2012). Bacterial pathogens of ventilator associated pneumonia in a tertiary referral hospital. *Tuberculosis and Respiratory Diseases*, *73*(1), 32-37.

Ching, C. K., Chen, S. P. L., Lee, H. H. C., Lam, Y. H., Ng, S. W., Chen, M. L., & Chan, T. Y. C. (2018). Adulteration of proprietary Chinese medicines and health products with undeclared drugs: experience of a tertiary toxicology laboratory in Hong Kong. *British journal of Clinical Pharmacology*, *84*(1), 172-178.

Cho, S. H., Strickland, I., Tomkinson, A., Fehringer, A. P., Gelfand, E. W. & Leung, D. Y. (2011). Preferential binding of *Staphylococcus aureus* to skin sites of Th2-

mediated inflammation in a murine model. *Journal of Investigative Dermatology*, *116*(5):658–663.

Chung, Y., Yang, X., Chang, S. H., Ma, L., Tian, Q., & Dong, C. (2006). Expression and regulation of IL-22 in the IL-17-producing CD4+ T lymphocytes. *Cell research*, *16*(11), 902.

Clinical and Laboratory Standard Institute (2017). CLSI Methods for Determining Bactericidal Activity of Antimicrobial Agents. Tentative Standards M26-T. Wayne: National Committee for Clinical Laboratory Standards. [Last accessed on 2017 Aug 25]. Available from: http://www.shop.clsi.org/site/Sample_pdf/M26A_sample.pdf .

Copp, B. R., & Pearce, A. N. (2007). Natural product growth inhibitors of *Mycobacterium tuberculosis. Natural Product Reports*, *24*(2), 278-297.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, *23*(2), 174-181.

Dahham, S., Tabana, M. Y., M. A. & Iqbal, M. A. 2015). "The anticancer, antioxidant and antimicrobial properties of the sesquiterpene *β*-caryophyllene from the essential oil of *Aquilaria crassna*," *Molecules*, vol. 20, no. 7, pp. 11808–11829.

Dahlen, J. R., Foster, D. C., & Kisiel, W. (1999). Inhibition of neutrophil elastase by recombinant human proteinase inhibitor 9. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1451*(2), 233-241.

Dambolena, J. S., Zunino, M. P., López, A. G., Rubinstein, H. R., Zygadlo, J. A., Mwangi, J. W., & Kariuki, S. T. (2010). Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*. *Innovative Food Science & Emerging Technologies*, *11*(2), 410-414.

Dancer, S. J. (2004). How antibiotics can make us sick: the less obvious adverse effects of antimicrobial chemotherapy. *The Lancet Infectious Diseases*, *4*(10), 611-619.

Daniel, V. N., Daniang, I. E., & Nimyel, N. D. (2011). Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in jos metropolis, plateau state, Nigeria. *International Journals of Engineering & Sciences*, *11*(6), 161-165.

Dasgupta, S., Das, S., Chawan, N. S., & Hazra, A. (2015). Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens

in a public tertiary teaching hospital of Eastern India. *Indian Journal of Critical Care Medicine, 19*(1), 14.

David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*, 23(3), 616-687.

Davis, K. A. (2006). Ventilator-associated pneumonia: a review. *Journal of Intensive Care Medicine*, *21*(4), 211-226.

De Francesco, A. S., Tanih, N. F., Samie, A., Guerrant, R. L., & Bessong, P. O. (2017). Antibiotic resistance patterns and beta-lactamase identification in *Escherichia coli* isolated from young children in rural Limpopo Province, South Africa: The MAL-ED cohort. *South African Medical Journal*, *107*(3), 205-214.

De Francesco, M. A., Ravizzola, G., Peroni, L., Negrini, R., & Manca, N. (2007). Urinary tract infections in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. *Medical Science Monitor*, *13*(6), 136-144.

De Groot, A. C., Nater, J. P., & Weyland, J. W. (1994). *Unwanted effects of cosmetics and drugs used in dermatology* (No. 282). Elsevier.

De Rosa, F. G., Garazzino, S., Audagnotto, S., Bargiacchi, O., Zeme, D. A., Gramoni, A., & Di Perri, G. (2008). SPIR01 and SPIR02: a two-year 1-day point prevalence multicenter study of infections in intensive care units in Piedmont, Italy. *Microbiologica-Quarterly Journal of Microbiological Sciences*, *31*(1), 81-88.

De Smet, P. A. (2004). Health risks of herbal remedies: an update. *Clinical Pharmacology & Therapeutics*, *76*(1), 1-17.

Deepak, V., Kasonga, A., Kruger, M. C., & Coetzee, M. (2016). Carvacrol inhibits osteoclastogenesis and negatively regulates the survival of mature osteoclasts. *Biological and Pharmaceutical Bulletin*, b16-00117.

Dehbanipour, R., Rastaghi, S., Sedighi, M., Maleki, N., & Faghri, J. (2016). High prevalence of multidrug-resistance uropathogenic *Escherichia coli* strains, Isfahan, Iran. *Journal of Natural Science, Biology, and Medicine*, *7*(1), 22.

Demirel, S., Tuzen, M., Saracoglu, S., & Soylak, M. (2008). Evaluation of various digestion procedures for trace element contents of some food materials. *Journal of hazardous materials*, *152*(3), 1020-1026.

Descotes, J. (2014). Introduction to immunotoxicology. CRC Press.

Devendran, G., & Balasubramanian, U. (2011). Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves. *Asian Journal of Plant Science and Research*, *1*(4), 44-48.

Diallo, A., Eklu-Gadegbeku, K., Amegbor, K., Agbonon, A., Aklikokou, K., Creppy, E., & Gbeassor, M. (2014). *In vivo* and *in vitro* toxicological evaluation of the hydroalcoholic leaf extract of *Ageratum conyzoides* L.(Asteraceae). *Journal of Ethnopharmacology*, *155*(2), 1214-1218.

Dias, V. C., Resende, J. A., Bastos, A. N., Bastos, L. Q. D. A., Bastos, V. Q. D. A., Bastos, R. V., & Silva, V. L. D. (2017). Epidemiological, Physiological, and Molecular Characteristics of a Brazilian Collection of Carbapenem-Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Microbial Drug Resistance*, *23*(7), 852-863.

Dilnessa, T., & Bitew, A. (2016). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. *BMC Infectious Diseases*, *16*(1), 398.

Dollah, M. A., Parhizkar, S., Latiff, L. A., & Hassan, M. H. B. (2013). Toxicity effect of *Nigella sativa* on the liver function of rats. *Advanced Pharmaceutical Bulletin*, *3*(1), 97.

Doshi, G. M., & Une, H. D. (2015). Screening of *Polyalthia Iongifolia* Leaves as Potential Immunomodulatory. *International Journal of Pharmacology*, *11*(2), 106-113.

Doughari, J. H., Human, I. S., Bennade, S., & Ndakidemi, P. A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, 3(11), 839-848.

EFSA (2013). Scientific opinion on dietary reference values for manganese, EFSA panel on dietetic products, nuturition and allergies (NDA), euiropean food safety authority, Parma, Italy. EFSA journal.

Egesie, U. G., Adelaiye, A. B., Ibu, J. O., & Egesie, O. J. (2006). Safety and hypoglycaemic properties of aqueous leaf extract of *Ocimum gratissimum* in streptozotocin induced diabetic rats. *Nigerian Journal of Physiological Sciences*, *21*(1-2).

El Hilaly, J., Israili, Z. H., & Lyoussi, B. (2004). Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. *Journal of Ethnopharmacology*, *91*(1), 43-50.

Elgert, K. D. (2009). *Immunology: understanding the immune system*. John Wiley & Sons.

Ericksen, B., Wu, Z., Lu, W., & Lehrer, R. I. (2005). Antibacterial activity and specificity of the six human α-defensins. *Antimicrobial Agents and Chemotherapy*, *49*(1), 269-275.

Eriksen, H. M., Chugulu, S., Kondo, S., & Lingaas, E. (2003). Surgical-site infections at Kilimanjaro Christian medical center. *Journal of Hospital Infection*, *55*(1), 14-20.

Ernst, C. M., Staubitz, P., Mishra, N. N., Yang, S. J., Hornig, G., Kalbacher, H., & Peschel, A. (2009). The bacterial defensin resistance protein MprF consists of separable domains for lipid lysinylation and antimicrobial peptide repulsion. *PLoS Pathogens*, *5*(11), e1000660.

Eswar, P., Devaraj, C. G., & Agarwal, P. (2016). Anti-microbial Activity of Tulsi {*Ocimum Sanctum* (Linn.)} Extract on a Periodontal Pathogen in Human Dental Plaque: An *In vitro* Study. *Journal of Clinical and Diagnostic Research: JCDR*, *10*(3), ZC53.

Fagbohun, E. D., Lawal, O. U., & Ore, M. E. (2012). The proximate, mineral and phytochemical analysis of the leaves of *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis* L. and their medicinal value. *American Journal of Food and Nutrition. 5*(3), 95-98.

Farquhar, J. W. (1996). Plant sterols: their biological effects in humans. *Handbook of Lipids in Human Nutrition*, 101-105.

Fathy, A., Abdelhafeez, R., Abdel-Hady, E. G., & Elhafez, S. A. A. (2013). Analysis of ventilator associated pneumonia (VAP) studies in Egyptian University Hospitals. *Egyptian Journal of Chest Diseases and Tuberculosis*, *62*(1), 17-25.

Fehr, J., Hatz, C., Soka, I., Kibatala, P., Urassa, H., Smith, T., & Widmer, A. (2006). Risk factors for surgical site infection in a Tanzanian district hospital: a challenge for the traditional National Nosocomial Infections Surveillance system index. *Infection Control & Hospital Epidemiology*, *27*(12), 1401-1404.

Feleke, Y., Mengistu, Y., & Enquselassie, F. (2007). Diabetic infections: clinical and bacteriological study at Tikur Anbessa Specialized University Hospital, Addis Ababa, Ethiopia. *Ethiopian Medical Journal*, *45*(2), 171-179.

Fofie, N., B., Y., Kiendrebeogo, M., Coulibaly, K., Sanogo, R. & Kone-bamba, D. (2016). Mineral Salt Composition and Secondary Metabolites of *Ocimum gratissimum* L., An Anti-hyperglycemic Plant. *Natural Products Chemistry and Research,* 4: 235.

Fontanay, S., Grare, M., Mayer, J., Finance, C., & Duval, R. E. (2008). Ursolic, oleanolic and betulinic acids: antibacterial spectra and selectivity indexes. *Journal of Ethnopharmacology*, *120*(2), 272-276.

Fosmire, G. J. (1990). Zinc toxicity. *The American Journal of Clinical Nutrition*, *51*(2), 225-227.

Foster, T. J. (2005). Immune evasion by staphylococci. *Nature Review Microbiology, 3,* 948–958.

Fournier, B., & Philpott, D. J. (2005). Recognition of *Staphylococcus aureus* by the innate immune system. *Clinical Microbiology Reviews*, *18*(3), 521-540.

Francomano, F., Caruso, A., Barbarossa, A., Fazio, A., La Torre, C., Ceramella, J., & Sinicropi, M. S. (2019). β-Caryophyllene: A Sesquiterpene with Countless Biological Properties. *Applied Sciences*, *9*(24), 5420.

Friedman, L., Alder, J. D., & Silverman, J. A. (2006). Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy*, *50*(6), 2137-2145.

Fritz, S. A., Tiemann, K. M., Hogan, P. G., Epplin, E. K., Rodriguez, M., Al-Zubeidi, D. N., & Hunstad, D. A. (2013). A serologic correlate of protective immunity against community-onset *Staphylococcus aureus* infection. *Clinical Infectious Diseases*, *56*(11), 1554-1561.

Fuchs, T. C., & Hewitt, P. (2011). Biomarkers for drug-induced renal damage and nephrotoxicity—an overview for applied toxicology. *The AAPS Journal*, *13*(4), 615-631.

Furr, S.R. (1998). Fundamentals of Immunobiology. In: *Pharmaceutical Microbiology*. Hugo, W.B., Russell, A.D. (Ed.), 6th Edn., Oxford: Blackwell Science Ltd., pp. 278-303.

Gaikwad, S. B., Mohan, D. G. K., & Reddy, K. J. (2011). *Moringa oleifera* leaves: Immunomodulation in wistar albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences, 3*(5), 975-1491.

García-Álvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F., Curran, M. D., & Parkhill, J. (2011). Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. *The Lancet Infectious Diseases*, *11*(8), 595-603.

Gastmeier, P., Sohr, D., Geffers, C., Behnke, M., & Rüden, H. (2007). Risk factors for death due to nosocomial infection in intensive care unit patients: findings from the Krankenhaus Infektions Surveillance System. *Infection Control & Hospital Epidemiology*, *28*(4), 466-472.

Gautam, M. K., & Goel, R. K. (2014). Toxicological study of *Ocimum sanctum* Linn leaves: hematological, biochemical, and histopathological studies. *Journal of Toxicology*, *2014*, Article ID 135654, 9 pages.

Ghasemzadeh, A., Jaafar, H. Z., Bukhori, M. F. M., Rahmat, M. H., & Rahmat, A. (2018). Assessment and comparison of phytochemical constituents and biological activities of bitter bean (Parkia speciosa Hassk.) collected from different locations in Malaysia. *Chemistry Central Journal*, *12*(1), 12.

Geginat, J., Paroni, M., Facciotti, F., Gruarin, P., Kastirr, I., Caprioli, F., & Abrignani, S. (2013, November). The CD4-centered universe of human T cell subsets. In *Seminars in Immunology*, *25*(4), 252-262.

Gellatly, S. L., & Hancock, R. E. (2013). *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathogens and Disease*, *67*(3), 159-173.

George, S., Nair, S. A., Venkataraman, R., & Baby, S. (2015). Chemical composition, antibacterial and anticancer activities of volatile oil of *Melicope denhamii* leaves. *Natural Product Research*, *29*(20), 1959-1962.

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., & Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences*, *105*(26), 9099-9104.

Girardi, M. (2007). Cutaneous perspectives on adaptive immunity. *Clinical Reviews in Allergy & Immunology*, 33(1-2), 4-14.

Golebiewska, E. M., & Poole, A. W. (2015). Platelet secretion: From haemostasis to wound healing and beyond. *Blood Reviews*, *29*(3), 153-162.

Gonzalez, R., Ballester, I., López-Posadas, R., Suárez, M. D., Zarzuelo, A., Martinez-Augustin, O., & Medina, F. S. D. (2011). Effects of flavonoids and other polyphenols on inflammation. *Critical Reviews in Food Science and Nutrition*, *51*(4), 331-362.

Gonzalez-Barca, E., Carratala, J., Mykietiuk, A., Fernandez-Sevilla, A., & Gudiol, F. (2001). Predisposing factors and outcome of *Staphylococcus aureus* bacteremia in neutropenic patients with cancer. *European Journal of Clinical Microbiology and Infectious Diseases*, *20*(2), 117–119.

Gordon, D. M. (2001). Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. *Microbiology*, *147*(5), 1079-1085.

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A., & Vernekar, S. N. (2010). Markers of renal function tests. *North American Journal of Medical Sciences*, *2*(4), 170.

Grimwood, K., Kyd, J. M., Owen, S. J., Massa, H. M., & Cripps, A. W. (2015). Vaccination against respiratory *Pseudomonas aeruginosa* infection. *Human Vaccines & Immunotherapeutics*, *11*(1), 14-20.

Guerrero, J. G., Martınez, J. G., & Isasa, M. T. (1998). Mineral nutrient composition of edible wild plants. *Journal of Food Composition and Analysis*, *11*(4), 322-328.

Hanif, M. A., Al-Maskari, M. Y., Al-Maskari, A., Al-Shukaili, A., Al-Maskari, A. Y., & Al-Sabahi, J. N. (2011). Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. *Journal of Medicinal Plants Research*, *5*(5), 751-757.

Heim, C. E., Vidlak, D., & Kielian, T. (2015). Interleukin-10 production by myeloid-derived suppressor cells contributes to bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection. *Journal of Leukocyte Biology*, *98*(6), 1003-1013.

Heroor, S., Beknal, A. K., & Mahurkar, N. (2013). Immunomodulatory activity of methanolic extracts of fruits and bark of *Ficus glomerata* Roxb. in mice and on human neutrophils. *Indian Journal of Pharmacology*, *45*(2), 130.

Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P. C. H., & Katan, M. B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, *342*(8878), 1007-1011.

Heyworth, P. G., Cross, A. R., and Curnutte, J. T. (2003). Chronic granulomatous disease. *Current Opinion in Immunology,* 15(5):578–584.

Hodges, A. M., & Agaba, S. (1997). Wound infection in a rural hospital: the benefit of a wound management protocol. *Tropical Doctor*, *27*(3), 174-175.

Hoffbrand, A. V., Moss, P. A. H., & Pettit, J. E. (2006). Essential Haematology, 2006. *Blackwell Publishing, Malden MA, pgs*, *5*, 249.

Holtfreter, S., Kolata, J. & Broker, B. M. (2010). Towards the immune proteome of *Staphylococcus aureus*—the anti-*S. aureus* antibody response. *International Journal of Medical Microbiology* 300:176–192.

Hong, J. H., Seah, S. W., & Xu, J. (2013). The root of ABA action in environmental stress response. *Plant cell reports*, *32*(7), 971-983.

Howden, B. P., Peleg, A. Y., & Stinear, T. P. (2014). The evolution of vancomycin intermediate *Staphylococcus aureus* (VISA) and heterogenous-VISA. *Infection, Genetics and Evolution*, *21*, 575-582.

Hruz, P., Zinkernagel, A. S., Jenikova, G., Botwin, G. J., Hugot, J. P., Karin, M., & Eckmann, L. (2009). NOD2 contributes to cutaneous defense against *Staphylococcus aureus* through α-toxin-dependent innate immune activation. *Proceedings of the National Academy of Sciences*, *106*(31), 12873-12878.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil *(Ocimum basilicum)* essential oils depends on seasonal variations. *Food Chemistry*, *108*(3), 986-995.

Hussain, I. & Khan, H. (2010). Investigation of Heavy Metals Content in Medicinal Plant, *Eclipta alba* L. *Environmental Pollution*, *32*(1), 5.

Hussain, I., Khan, F., Khan, I., Khan, L. & Wali-Ullah, (2006). Determination of Heavy metals in medicinal plants. *Journal of Chemical Society of Pakistan, 28*(4), 347-351.

Imelouane, B., Tahri, M., Elbastrioui, M., Aouinti, F., & Elbachiri, A. (2011). Mineral contents of some medicinal and aromatic plants growing in eastern Morocco. *Journal of Material and Environmental Science*, *2*(2), 104-111.

Indrayan, A. K., Sharma, S., Durgapal, D., Kumar, N., & Kumar, M. (2005). Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Science*, *89*(7), 1252-1255.

IOM (05). Dietary reference intake for Ca, P, Mg, Vit D, and fluoride. Food and nutrition board, national academy of sciences; the national academy press; Wahington, DC, USA.

IOM, (1997). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary reference intakes. In *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academies Press (US).

IOM, (2005). Panel on Dietary Reference Intakes for Electrolytes, & Water. (2005). *DRI, dietary reference intakes for water, potassium, sodium, chloride, and sulfate*. National Academy Press.

Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, *87*(4), 581-586.

Ito, T., Hiramatsu, K., Tomasz, A., De Lencastre, H., Perreten, V., Holden, M. T., & Zhang, K. (2012). Guidelines for reporting novel mecA gene homologues. *Antimicrobial Agents and Chemotherapy*, *56*(10), 4997-4999.

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., & Littman, D. R. (2006). The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*, *126*(6), 1121-1133.

Jaillon, S., Galdiero, M. R., Del Prete, D., Cassatella, M. A., Garlanda, C., & Mantovani, A. (2013, July). Neutrophils in innate and adaptive immunity. *Seminars in Immunopathology, 35*(4), 377-394.

Jansen, W. T. M., Beitsma, M. M., Koeman, C. J., Van Wamel, W. J. B., Verhoef, J., & Fluit, A. C. (2006). Novel mobile variants of staphylococcal cassette chromosome mec in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *50*(6), 2072-2078.

Jayaraman, P., Sakharkar, M. K., Lim, C. S., Tang, T. H., & Sakharkar, K. R. (2010). Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. *International Journal of Biological Sciences*, *6*(6), 556-568.

Jayasri, M. A., Lazar, M., & Radha, A. (2009). A report on the antioxidant activity of leaves and rhizomes of Costus pictus D. Don. *International Journal of Integrative Biology*, *5*(1), 20-26.

Jeba, R. C., & Rameshkumar, G. (2013). Comparative study of immunomodulatory activity of ocimum species in mice. *International Journal of Pharmaceutical Science Research, 4(9),* 3518-3523.

Jefferies, J. M. C., Cooper, T., Yam, T., & Clarke, S. C. (2012). *Pseudomonas aeruginosa* outbreaks in the neonatal intensive care unit–a systematic review of risk factors and environmental sources. *Journal of Medical Microbiology*, *61*(8), 1052-1061.

Jevons, M. P., & Parker, M. T. (1964). The evolution of new hospital strains of *Staphylococcus aureus*. *Journal of Clinical Pathology*, *17*(3), 243-250.

Jordan, S. A., Cunningham, D. G., & Marles, R. J. (2010). Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology*, *243*(2), 198-216.

Joseph, N. M., Sistla, S., Dutta, T. K., Badhe, A. S., Rasitha, D., & Parija, S. C. (2012). Outcome of ventilator-associated pneumonia: impact of antibiotic therapy and other factors. *The Australasian Medical Journal*, *5*(2), 135.

Joshi, R. K. (2014). Chemical composition and antimicrobial activity of the essential oil of *Ocimum basilicum* L. (sweet basil) from Western Ghats of North West Karnataka, India. *Ancient Science of Life*, *33*(3), 151.

Junqueira, L. C., Carneiro, J., & Kelley, R. O. (2006). Basic Histology. A Lange Medical Book. Appleton and Lange (7th edn): 72-125.

Kajiya, K., Hojo, H., Suzuki, M., Nanjo, F., Kumazawa, S., & Nakayama, T. (2004). Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *Journal of Agricultural and Food Chemistry*, *52*(6), 1514-1519.

Kale, O. E., & Awodele, O. (2016). Safety evaluation of Bon-santé cleanser® polyherbal in male Wistar rats. *BMC Complementary and Alternative Medicine*, *16*(1), 188.

Kale, O. E., Akinpelu, O. B., Bakare, A. A., Yusuf, F. O., Gomba, R., Araka, D. C., & Odutola, O. (2018). Five traditional Nigerian Polyherbal remedies protect against high fructose fed, Streptozotocin-induced type 2 diabetes in male Wistar rats. *BMC Complementary and Alternative Medicine*, *18*(1), 160.

Kale, O. E., Awodele, O., & Akindele, A. J. (2019). *Acridocarpus smeathmannii* (DC.) Guill. & Perr. Root enhanced reproductive behavior and sexual function in male wistar rats: Biochemical and pharmacological mechanisms. *Journal of Ethnopharmacology*, *230*, 95-108.

Kale, O. E., Oyesola, T. O., & Raji, F. S. (2018). Celecoxib, a cyclooxygenase-2 inhibitor, offers chemoprevention against reproductive and neurobehavioural abnormalities induced by atrazine in male Wistar rats. *Environmental Toxicology and Pharmacology*, *58*, 84-97.

Kar, H. K., Bhatia, V. N., Kumar, C. H., Sirumban, P., & Roy, R. G. (1986). Evaluation of levamisole, an immunopotentiator, in the treatment of lepromatous leprosy. *Indian Journal of Leprosy*, *58*(4), 592-600.

Karauzum, H., & Datta, S. K. (2016). Adaptive immunity against *Staphylococcus aureus*. *Current Topics in Microbiology and Immunology, 409*: 419-439.

Katoch, K. (1996). Immunotherapy of leprosy. *Indian Journal of Leprosy*, *68*(4), 349-361.

Kawai, T. & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature Immunology*. 11, 373–384.

Kawashima, L. M., & Soares, L. M. V. (2003). Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. *Journal of Food Composition and Analysis*, *16*(5), 605-611.

Kaya, I., Yiğit, N., & Benli, M. (2008). Antimicrobial activity of various extracts of *Ocimum basilicum* L. and observation of the inhibition effect on bacterial cells by use of scanning electron microscopy. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(4), 363-369.

Kelesidis, T., Humphries, R., Uslan, D. Z., & Pegues, D. A. (2011). Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. *Clinical Infectious Diseases*, *52*(2), 228-234.

Kiendrebeogo, M., Coulibaly, K., Sanogo, R., & Kone-Bamba, D. (2016). Mineral salt composition and secondary metabolites of *Ocimum gratissimum* L., an antihyperglycemic plant. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 425.

Kim, C., Milheiriço, C., Gardete, S., Holmes, M. A., Holden, M. T., de Lencastre, H., & Tomasz, A. (2012). Properties of a novel PBP2A protein homolog from *Staphylococcus aureus* strain LGA251 and its contribution to the β-lactam-resistant phenotype. *Journal of Biological Chemistry*, *287*(44), 36854-36863.

Kim, H. K., Thammavongsa, V., Schneewind, O., & Missiakas, D. (2012). Recurrent infections and immune evasion strategies of *Staphylococcus aureus*. *Current Opinion in Microbiology*, *15*(1), 92-99.

Kim, J., Lee, J. J., Kim, J., Gardner, D., & Beachy, P. A. (2010). Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. *Proceedings of the National Academy of Sciences*, *107*(30), 13432-13437.

Kim, O. Y., Hong, B. S., Park, K. S., Yoon, Y. J., Choi, S. J., Lee, W. H., & Gho, Y. S. (2013). Immunization with *Escherichia coli* outer membrane vesicles protects bacteria-induced lethality via Th1 and Th17 cell responses. *The Journal of Immunology*, *190*(8), 4092-4102.

Kim, W. K., Song, S. Y., Oh, W. K., Kaewsuwan, S., Tran, T. L., Kim, W. S., & Sung, J. H. (2013). Wound-healing effect of ginsenoside Rd from leaves of *Panax ginseng* via cyclic AMP-dependent protein kinase pathway. *European Journal of Pharmacology*, *702*(1-3), 285-293.

Kim, W., Zhu, W., Hendricks, G. L., Van Tyne, D., Steele, A. D., Keohane, C. E., & Lee, K. (2018). A new class of synthetic retinoid antibiotics effective against bacterial persisters. *Nature*, *556*(7699), 103-107.

Kimang'a, A., Gikunju, J., Kariuki, D., & Ogutu, M. (2016). Safety and analgesic properties of ethanolic extracts of *Toddalia asiatica* (L) Lam.(rutaceae) used for central and peripheral pain management among the east african ethnic communities. *Ethiopian Journal of Health Sciences*, *26*(1), 55-66.

Kobayashi, S. D. & Deleo, F. R. (2011). A MRSA-terious enemy among us: boosting MRSA vaccines. *Nature Medicine* 17:168–169.

Kocić-Tanackov, S., Dimić, G., Lević, J., Tanackov, I., & Tuco, D. (2011). Antifungal activities of basil (*Ocimum basilicum* L.) extract on Fusarium species. *African Journal of Biotechnology*, *10*(50), 10188-10195.

Koh, A. Y., Priebe, G. P., Ray, C., Van Rooijen, N., & Pier, G. B. (2009). Inescapable need for neutrophils as mediators of cellular innate immunity to acute *Pseudomonas aeruginosa* pneumonia. *Infection and Immunity*, *77*(12), 5300-5310.

Koigi-Kamau, R., Kabare, L. W., & Wanyoike-Gichuhi, J. (2005). Incidence of wound infection after caesarean delivery in a district hospital in central Kenya. *East African Medical Journal*, *82*(7), 357-361.

Kolanjiappan, K., Manoharan, S., & Kayalvizhi, M. (2002). Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. *Clinica Chimica Acta*, *326*(1), 143-149.

Kolata, J. B., Kühbandner, I., Link, C., Normann, N., Vu, C. H., Steil, L., & Bröker, B. M. (2015). The fall of a dogma? Unexpected high T-cell memory response to *Staphylococcus aureus* in humans. *The Journal of Infectious Diseases*, *212*(5), 830-838.

Kotisso, B., & Aseffa, A. (1998). Surgical wound infection in a teaching hospital in Ethiopia. *East African Medical Journal*, *75*(7), 402-405.

Krishna, S., & Miller, L. S. (2012). Innate and adaptive immune responses against *Staphylococcus aureus* skin infections. *Seminars in Immunopathology*, 34(2), 261-280.

Kubo, I., Xiao, P., & Fujita, K. I. (2002). Anti-MRSA activity of alkyl gallates. *Bioorganic & Medicinal Chemistry Letters*, *12*(2), 113-116.

Kuby J. (1997). Immunology. 3rd ed. WH Freeman and Company; New York; p. 436.

Kuehnert, M. J., Hill, H. A., Kupronis, B. A., Tokars, J. I., Solomon, S. L., & Jernigan, D. B. (2005). Methicillin-resistant-*Staphylococcus aureus* hospitalizations, United States. *Emerging Infectious Diseases*, 11(6), 868-872.

Kuete, V. (2014). Toxic Plants Used in African Traditional Medicine. In *Toxicological Survey of African Medicinal Plants* (pp. 135-180). eBook ISBN: 9780128004753

Kumar, S. H., De, A. S., Baveja, S. M., & Gore, M. A. (2012). Prevalence and risk factors of metallo β-lactamase producing *Pseudomonas aeruginosa* and Acinetobacter species in burns and surgical wards in a tertiary care hospital. *Journal of Laboratory Physicians*, *4*(1), 39.

Kumar, V., Sharma, A., Dhunna, G., Chawla, A., Bhardwaj, R., & Thukral, A. K. (2017). A tabulated review on distribution of heavy metals in various plants. *Environmental Science and Pollution Research*, *24*(3), 2210-2260.

Kupper, T. S., & Fuhlbrigge, R. C. (2004). Immune surveillance in the skin: mechanisms and clinical consequences. *Nature Reviews Immunology*, *4*(3), 211-222.

Kurdi, A., Hassan, K., Venkataraman, B., & Rajesh, M. (2018). Nootkatone confers hepatoprotective and anti- fibrotic actions in a murine model of liver fibrosis by suppressing oxidative stress, inflammation, and apoptosis. *Journal of Biochemical and Molecular Toxicology*, *32*(2), e22017.

Lai, Z. R., Ho, Y. L., Huang, S. C., Huang, T. H., Lai, S. C., Tsai, J. C., & Chang, Y. S. (2011). Antioxidant, anti-inflammatory and antiproliferative activities of

Kalanchoe gracilis (L.) DC stem. *The American Journal of Chinese Medicine*, *39*(06), 1275-1290.

Las Heras, B., Rodriguez, B., Bosca, L., & Villar, A. M. (2003). Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. *Current Topics in Medicinal Chemistry*, *3*(2), 171-185.

Lavoie, E. G., Wangdi, T., & Kazmierczak, B. I. (2011). Innate immune responses to *Pseudomonas aeruginosa* infection. *Microbes and Infection*, *13*(14), 1133-1145.

Lee, H., & Lee, D. G. (2015). Mode of action of bioactive phytochemicals, plant secondary metabolites, possessing antimicrobial properties. *The Battle against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs (A. Méndez-Vilas, Ed.)*

Leegaard, T. M., Caugant, D. A., Frøholm, L. O., & Høiby, E. A. (2000). Apparent differences in antimicrobial susceptibility as a consequence of national guidelines. *Clinical Microbiology and Infection*, *6*(6), 290-293.

Legras, A., Malvy, D., Quinioux, A. I., Villers, D., Bouachour, G., Robert, R., & Thomas, R. (1998). Nosocomial infections: prospective survey of incidence in five French intensive care units. *Intensive Care Medicine*, *24*(10), 1040-1046.

Lehrer, R. I. (2007). Multispecific myeloid defensins. *Current Opinion in Hematolology*, 14(1), 16–21

Leonhardt, R. H., & Berger, R. G. (2014). Nootkatone. In *Biotechnology of Isoprenoids* (pp. 391-404). Springer, Cham.

Ley, K., Laudanna, C., Cybulsky, M. I., & Nourshargh, S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nature Reviews Immunology*, *7*(9), 678-689.

Liang, S. C., Tan, X. Y., Luxenberg, D. P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., & Fouser, L. A. (2006). Interleukin (IL)-22 and IL-17 are co-expressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *Journal of Experimental Medicine*, *203*(10), 2271-2279.

Liao, X., Xie, Y. M., Robinson, N., & Wang, Y. Y. (2017). To establish a body of evidence on safety for postmarketing Chinese medicine: A new research paradigm. *Chinese Journal of Integrative Medicine*, *23*(3), 226-232.

Lieleg, O., Caldara, M., Baumgärtel, R., & Ribbeck, K. (2011). Mechanical robustness of *Pseudomonas aeruginosa* biofilms. *Soft Matter*, *7*(7), 3307-3314.

Lima, B., López, S., Luna, L., Agüero, M. B., Aragón, L., Tapia, A., & Feresin, G. E. (2011). Essential Oils of Medicinal Plants from the Central Andes of Argentina: Chemical Composition, and Antifungal, Antibacterial, and Insect- Repellent Activities. *Chemistry & biodiversity*, *8*(5), 924-936.

Lisiewska, Z., Gębczyński, P., Bernaś, E., & Kmiecik, W. (2009). Retention of mineral constituents in frozen leafy vegetables prepared for consumption. *Journal of Food Composition and Analysis*, *22*(3), 218-223.

Liu, A. G., Volker, S. E., Jeffery, E. H., & Erdman Jr, J. W. (2009). Feeding tomato and broccoli powders enriched with bioactives improves bioactivity markers in rats. *Journal of Agricultural and Food Chemistry*, *57*(16), 7304-7310.

Liu, M., Dai, Y., Li, Y., Luo, Y., Huang, F., Gong, Z., & Meng, Q. (2008). Madecassoside isolated from *Centella asiatica* herbs facilitates burn wound healing in mice. *Planta Medica*, *74*(08), 809-815.

Llata, E., Gaynes, R. P., Fridkin, S., & Weinstein, R. A. (2009). Measuring the scope and magnitude of hospital-associated infection in the United States: the value of prevalence surveys. *Clinical Infectious Diseases*, *48*(10), 1434-1440.

Long, K. S., & Vester, B. (2012). Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrobial Agents and Chemotherapy*, *56*(2), 603-612.

Lopez, E., Bermejo, N., Berna-Erro, A., Alonso, N., Salido, G. M., Redondo, P. C., & Rosado, J. A. (2015). Relationship between calcium mobilization and platelet α- and δ-granule secretion. A role for TRPC6 in thrombin-evoked δ-granule exocytosis. *Archives of Biochemistry and Biophysics*, *585*(1), 75-81.

Loureiro, M. M., De Moraes, B. A., Mendonça, V. L. F., Quadra, M. R. R., Pinheiro, G. S., & Asensi, M. D. (2002). *Pseudomonas aeruginosa*: study of antibiotic resistance and molecular typing in hospital infection cases in a neonatal intensive care unit from Rio de Janeiro City, Brazil. *Memórias do Instituto Oswaldo Cruz*, *97*(3), 387-394.

Ma, J., Li, N., Liu, Y., Wang, C., Liu, X., Chen, S., & Wang, F. (2017). Antimicrobial resistance patterns, clinical features, and risk factors for septic shock and death of nosocomial *E. coli* bacteremia in adult patients with hematological disease: A monocenter retrospective study in China. *Medicine*, *96*(21), e6959.

Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., & Ray, S. M. (2014). Multistate point-prevalence survey of health care–associated infections. *New England Journal of Medicine*, *370*(13), 1198-1208.

Mahamood K, Yaqoob U, Bajwa R. (2008). Antibacterial activity of essential oil of *Ocimum sanctum* (L) Mycopath, 6:63–65.

Mahlangeni, N. T., Moodley, R., & Jonnalagadda, S. B. (2014). Elemental composition of *Cyrtanthus obliquus* and *Lippia javanica* used in South African herbal tonic, Imbiza. *Arabian Journal of Chemistry. 11*(1), 128-136

Mahmoud, A. M. (2013). Hematological alterations in diabetic rats-role of adipocytokines and effect of citrus flavonoids. *EXCLI Journal*, *12*, 647.

Makare, N., Bodhankar, S., & Rangari, V. (2001). Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. *Journal of Ethnopharmacology*, *78*(2), 133-137.

Malacarne, P., Boccalatte, D., Acquarolo, A., Agostini, F., Anghileri, A., Giardino, M., & Rossi, C. (2010). Epidemiology of nosocomial infection in 125 Italian intensive care units. *Minerva Anestesiologica*, *76*(1), 13.

Mamuye, Y. (2016). Antibiotic Resistance Patterns of Common Gram-negative Uropathogens in St. Paul's Hospital Millennium Medical College. *Ethiopian Journal of Health Sciences*, *26*(2), 93-100.

Mani, R., Murugan, P., Velpandian Venkatachalapathy, D. S., & Vellaiyan, B. (2015). An Overview of the Effect of Siddha Immunomodulators against Various Diseases and Infections. *European Journal of Pharmaceutical and Medical Research, 2*(6), 120-128.

Maret, W. (2013). Zinc biochemistry: from a single zinc enzyme to a key element of life. *Advances in Nutrition*, *4*(1), 82-91.

Marra, A. R., Camargo, L. F. A., Pignatari, A. C. C., Sukiennik, T., Behar, P. R. P., Medeiros, E. A. S., & Brites, C. (2011). Nosocomial bloodstream infections in Brazilian hospitals: analysis of 2,563 cases from a prospective nationwide surveillance study. *Journal of Clinical Microbiology*, *49*(5), 1866-1871.

Martin, S., & Griswold, W. (2009). Human health effects of heavy metals. *Environmental Science and Technology Briefs for Citizens*, *15*, 1-6.

Matos, I., Bento, A. F., Marcon, R., Claudino, R. F., & Calixto, J. B. (2013). Preventive and therapeutic oral administration of the pentacyclic triterpene α, β-amyrin ameliorates dextran sulfate sodium-induced colitis in mice: the relevance of cannabinoid system. *Molecular Immunology*, *54*(3-4), 482-492.

Mattson, M. P. (2008). Dietary factors, hormesis and health. *Ageing Research Reviews*, *7*(1), 43-48.

Mediratta, P. K., Sharma, K. K., & Singh, S. (2002). Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. *Journal of Ethnopharmacology*, *80*(1), 15-20.

Mehreen, A., Waheed, M., Liaqat, I., & Arshad, N. (2016). Phytochemical, antimicrobial, and toxicological evaluation of traditional herbs used to treat sore throat. *BioMed Research International*, Article ID 8503426, 9 pages, 2016. https://doi.org/10.1155/2016/8503426.

Melo, C. M., Carvalho, K. M. M. B., de Sousa Neves, J. C., Morais, T. C., Rao, V. S., Santos, F. A., & Chaves, M. H. (2010). α, β-amyrin, a natural triterpenoid ameliorates L-arginine-induced acute pancreatitis in rats. *World Journal of Gastroenterology: WJG*, *16*(34), 4272.

Mengel, K. and Kirkby, E., A. (1987). Principles of Plant Nutrition, 4th Edition (Revised), International Potash Institute, Switzerland, 501.

Miller, L. S., O'Connell, R. M., Gutierrez, M. A., Pietras, E. M., Shahangian, A., Gross, C. E., & Modlin, R. L. (2006). MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against *Staphylococcus aureus*. *Immunity*, *24*(1), 79-91.

Mishra P., Mishra S. (2011). Study of antibacterial activity of *Ocimum sanctum* extract against Gram positive and Gram negative bacteria. *American Journal of Food Technology*, 6(1), 336–341

Mlitan, A. M., Sasi, M. S., & Alkherraz, A. M. (2014). Proximate and minor mineral content in some selected basil leaves of *Ocimum gratissimum* L, in Libya. *International Journal of Chemical Engineering and Applications*, *5*(6), 502.

Molne, L., Verdrengh, M. & Tarkowski, A. (2000). Role of neutrophil leukocytes in cutaneous infection caused by *Staphylococcus aureus*. *Infection and Immunology* 68, 6162–6167 (2000).

Moody, R., antaram Joshi, S. H., & Chaney, W. (1995). Use of lectins as diagnostic and therapeutic tools for cancer. *Journal of Pharmacological and Toxicological Methods*, *33*(1), 1-10.

Morgun, A., Dzutsev, A., Dong, X., Greer, R. L., Sexton, D. J., Ravel, J., & Shulzhenko, N. (2015). Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. *Gut*, 64(11):1732-43.

Muenzer, J. T., Davis, C. G., Chang, K., Schmidt, R. E., Dunne, W. M., Coopersmith, C. M., & Hotchkiss, R. S. (2010). Characterization and

modulation of the immunosuppressive phase of sepsis. *Infection and Immunity*, *78*(4), 1582-1592.

MuKherjee, M., BaSu, S., MuKherjee, S. K., & MajuMder, M. (2013). Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *Escheriachia coli* which were isolated from hospitalized patients in Kolkata, India. *Journal of Clinical and Diagnostic Research, 7*(3), 449.

Mukinda, J. T., & Eagles, P. F. (2010). Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female mice and rats. *Journal of Ethnopharmacology*, *128*(1), 236-240.

Mukinda, J. T., & Syce, J. A. (2007). Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Journal of Ethnopharmacology*, *112*(1), 138-144.

Mulu, A., Moges, F., Tessema, B., & Kassu, A. (2006). Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, Northwest Ethiopia. *Ethiopian Medical Journal*, *44*(2), 125-131.

Murphy, A. G., O'Keeffe, K. M., Lalor, S. J., Maher, B. M., Mills, K. H., & McLoughlin, R. M. (2015). Correction: *Staphylococcus aureus* Infection of mice expands a population of memory γδ T cells that are protective against subsequent infection. *The Journal of Immunology*, *194*(9), 4588-4588.

Mutch, R. S., & Hutson, P. R. (1991). Levamisole in the adjuvant treatment of colon cancer. *Clinical Pharmacy*, *10*(2), 95-109.

Nabavi, S. F., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E., Daglia, M., & Nabavi, S. M. (2015). Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. *Nutrients*, *7*(9), 7729-7748.

Nabulo, G., Oryem-Origa, H., & Diamond, M. (2006). Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. *Environmental Research*, *101*(1), 42-52.

Naik, H. R. P., Naik, H. S. B., Naik, T. R. R., Naika, H. R., Gouthamchandra, K., Mahmood, R., & Ahamed, B. K. (2009). Synthesis of novel benzo [h] quinolines: wound healing, antibacterial, DNA binding and *in vitro* antioxidant activity. *European Journal of Medicinal Chemistry*, *44*(3), 981-989.

Namrata, C. (2013). Structure of Hemoglobin-An Overview. Biochemistry for Medics. Adapted from http://www.namrata.co/structure-of-hemoglobin-an-overview/ on 15th July, 2017.

Natarajan, B., Paulsen, B. S., & Pushpangadan, P. (1999). An ethnopharmacological study from the Coimbatore district, Tamil Nadu, India: traditional knowledge compared with modern biological science. *Pharmaceutical Biology*, *37*(5), 378-390.

Nazar, R., Iqbal, N., Masood, A., Khan, M. I. R., Syeed, S., & Khan, N. A. (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *American Journal of Plant Sciences*, *3*(10), 1476.

Nejad, S. B., Allegranzi, B., Syed, S. B., Ellis, B., & Pittet, D. (2011). Health-care-associated infection in Africa: a systematic review. *Bulletin of the World Health Organization*, *89*(10), 757-765.

Nemmar, A., Al-Salam, S., Beegam, S., Yuvaraju, P., Hamadi, N., & Ali, B. H. (2018). *In vivo* protective effects of nootkatone against particles-induced lung injury caused by diesel exhaust is mediated via the NF-κB pathway. *Nutrients*, *10*(3), 263.

Nfambi, J., Bbosa, G. S., Sembajwe, L. F., Gakunga, J., & Kasolo, J. N. (2015). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in Wistar albino rats. *Journal of Basic and Clinical Physiology and Pharmacology*, *26*(6), 603-611.

Nile, S. H., & Khobragade, C. N. N. (2009). Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *Journal of Medicinal Plants*, *8*(5).

Ning, B. T., Zhang, C. M., Liu, T., Ye, S., Yang, Z. H., & Chen, Z. J. (2013). Pathogenic analysis of sputum from ventilator-associated pneumonia in a pediatric intensive care unit. *Experimental and Therapeutic Medicine*, *5*(1), 367-371.

Njeru, S. N., Obonyo, M., Nyambati, S., Ngari, S., Mwakubambanya, R., & Mavura, H. (2016). Antimicrobial and cytotoxicity properties of the organic solvent fractions of *Clerodendrum myricoides* (Hochst.) R. Br. ex Vatke: Kenyan traditional medicinal plant. *Journal of Intercultural Ethnopharmacology*, *5*(3), 226.

Nobandegani, N. M., Mahmoudi, S., Pourakbari, B., Sadeghi, R. H., Sani, M. N., Farahmand, F., & Mamishi, S. (2016). Antimicrobial susceptibility of microorganisms isolated from sputum culture of patients with cystic fibrosis: Methicillin-resistant *Staphylococcus aureus* as a serious concern. *Microbial Pathogenesis*, *100*, 201-204.

Nogueira, A. O., Oliveira, Y. I. S., Adjafre, B. L., de Moraes, M. E. A., & Aragao, G. F. (2019). Pharmacological effects of the isomeric mixture of alpha and beta amyrin from *Protium heptaphyllum*: a literature review. *Fundamental & Clinical Pharmacology*, *33*(1), 4-12.

Novosad, S. A. (2016). Vital signs: epidemiology of sepsis: prevalence of health care factors and opportunities for prevention. *Morbidity and Mortality Weekly Report*, *65*.

Núñez, I., Arranz, J. C., Rivas, C., A., Mendonça, P., Pérez, K., Sánchez, M., Cortinhas, B., Silva, C., Carvalho, G., & Queiroz, M. (2017). Chemical Composition and Toxicity of *Ocimum sanctum* L. Var. *Cubensis* Essential Oil Up-Growing in the Eastern of Cuba. *International Journal of Pharmacognosy and Phytochemical Research, 9*(7); 1021-1028.

Nyamai, D. W., Mawia, A. M., Wambua, F. K., Njoroge, A., & Matheri, F. (2015). Phytochemical profile of *Prunus africana* stem bark from Kenya. *Journal of Pharmacognosy and Natural Products*, *1*(1), 2-8.

O'malley, T., Langhorne, P., Elton, R. A., & Stewart, C. (1995). Platelet size in stroke patients. *Stroke*, *26*(6), 995-999.

Oboh, G. (2010). Antioxidant and Antimicrobial properties of ethanolic extracts of *Ocimum gratissimum* leaves. *Journal of Pharmacology and Toxicology, 1*(5):396–302.

Oboh, G., & Rocha, J. B. T. (2007). Polyphenols in red pepper [*Capsicum annuum* var. aviculare (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *European Food Research and Technology*, *225*(2), 239-247.

Odebisi-Omokanye, M. B., Agbabiaka, T. O., Zakariyah, R. F., & Sanya, O. (2016). Antibacterial effect of *Ocimum gratissimum* against bacteria from paediatric diarrhoeal stool samples in the tropics. *Journal of Science and Technology (Ghana)*, *36*(3), 64-74.

OECD, (1998). OECD Principles of Good Laboratory Practice, OECD Publishing, Paris, 1998.

OECD, T. N. (2008). Repeated Dose 28-day Oral Toxicity Study in Rodents. *OECD Guidelines for the Testing of Chemicals, Section*, *4*.

Ofem, O. E., Ani, E. J., & Eno, A. E. (2012). Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rats. *International Journal of Applied and Basic Medical Research*, *2*(1), 38.

Ogundipe, D. J., Akomolafe, R. O., Sanusi, A. A., Imafidon, C. E., Olukiran, O. S., & Oladele, A. A. (2016). Effects of two weeks administration of *Ocimum gratissimum* leaf on feeding pattern and markers of renal function in rats treated with gentamicin. *Egyptian Journal of Basic and Applied Sciences*, *3*(3), 219-231.

Ojo, O. A., Oloyede, O. I., Olarewaju, O. I., Ojo, A. B., Ajiboye, B. O., & Onikanni, S. A. (2013). Toxicity studies of the crude aqueous leaves extracts of *Ocimum gratissimum* in albino Rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *6*, 434-439.

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., & Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet Infectious Diseases*, *5*(8), 481-493.

Olatunji, F. O., Fadeyi, A., Ayanniyi, A. A., & Akanbi 2nd, A. A. (2007). Non-gonococcal bacterial agents of conjunctivitis and their antibiotic susceptibility patterns in Ilorin, Nigeria. *African Journal of Medicine and Medical Sciences*, *36*(3), 243-247.

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., & Dorato, M. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology*, *32*(1), 56-67.

Omojate Godstime, C., Enwa Felix, O., Jewo Augustina, O., & Eze Christopher, O. (2014). Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens–a review. *Journal of Pharmaceutical, Chemical and Biological Sciences*, *2*(2), 77-85.

Omosa, L. K., Amugune, B., Ndunda, B., Milugo, T. K., Heydenreich, M., Yenesew, A., & Midiwo, J. O. (2014). Antimicrobial flavonoids and diterpenoids from *Dodonaea angustifolia*. *South African Journal of Botany*, *91*, 58-62.

Omuse, G., Kabera, B., & Revathi, G. (2014). Low prevalence of methicillin resistant *Staphylococcus aureus* as determined by an automated identification system in two private hospitals in Nairobi, Kenya: a cross sectional study. *BMC Infectious Diseases*, *14*(1), 669.

Ong, P. Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., & Leung, D. Y. (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New England Journal of Medicine*, *347*(15), 1151-1160.

Onwuka, N. A. (2016). *Evaluation of the Immunomodulatory Activity of Hoslundia Opposita Vahl (Lamiaceae) Leaf Extract* (Doctoral dissertation).

O'Shea, J. J. & Paul, W. E. (2010). Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science* 327:1098–1102.

Ouyang, W., Kolls, J. K., & Zheng, Y. (2008). The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity*, *28*(4), 454-467.

Pachkore, G. L., & Dhale, D. A. (2012). Phytochemicals, Vitamins and Mineral s Content of Three Ocimum Species. *International Journal of Science Innovations and Discoveries*, *2*(1), 201-207.

Pallabi, D. E., Dasgupta, S. C., & Gomes, A. (1998). Immunopotentiating and immunoprophylactic activities of lmmue 21, a polyherbal product. *Indian Journal of Pharmacology*, *30*(3), 163.

Palmer, K. L., Daniel, A., Hardy, C., Silverman, J., & Gilmore, M. S. (2011). Genetic basis for daptomycin resistance in enterococci. *Antimicrobial Agents and Chemotherapy*, *55*(7), 3345-3356.

Panigrahy, B., Grumbles, L. C., Millar, D., Naqi, S. A., & Hall, C. F. (1979). Antibiotic-induced immunosuppression and levamisole-induced immunopotentiation in turkeys. *Avian Diseases*, 401-408.

Park, K. I., Kang, S. R., Park, H. S., Lee, D. H., Nagappan, A., Kim, J. A., & An, S. J. (2012). Regulation of proinflammatory mediators via NF-κB and p38 MAPK-dependent mechanisms in RAW 264.7 macrophages by polyphenol components isolated from Korea *Lonicera japonica* Thunb. *Evidence-Based Complementary and Alternative Medicine*, *2012*.

Pasman, W. J., Heimerikx, J., Rubingh, C. M., van den Berg, R., O'Shea, M., Gambelli, L., & Mennen, L. I. (2008). The effect of Korean pine nut oil on *in vitro* CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. *Lipids in Health and Disease*, *7*(1), 10.

Patra, K., Bose, S., Sarkar, S., Rakshit, J., Jana, S., Mukherjee, A., & Bhattacharjee, S. (2012). Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. *Chemico-biological Interactions*, *195*(3), 231-239.

Pattanayak, P., Behera, P., Das, D., & Panda, S. K. (2010). *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy Reviews*, *4*(7), 95.

Pereira, D. M., Correia-da-Silva, G., Valentão, P., Teixeira, N., & Andrade, P. B. (2014). Anti-inflammatory effect of unsaturated fatty acids and ergosta-7, 22-dien-3-ol from *Marthasterias glacialis*: Prevention of CHOP-mediated ER-stress and NF-κB activation. *PLoS One*, *9*(2), e88341.

Peres, A. G., Stegen, C., Li, J., Xu, A. Q., Levast, B., Surette, M. G., & Madrenas, J. (2015). Uncoupling of pro-and anti-inflammatory properties of *Staphylococcus aureus. Infection and Immunity,* 83(4), 1587-1597.

Périchon, B., & Courvalin, P. (2009). VanA-type vancomycin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *53*(11), 4580-4587.

Phillipson, J. D. (2007). Phytochemistry and pharmacognosy. *Phytochemistry*, *68*(22), 2960-2972.

Pinto, D., Fernandes, A., Fernandes, R., Mendes, I., Pereira, S., Vinha, A., ... & Machado, M. (2011). Determination of heavy metals and other indicators in waters, soils and medicinal plants from Ave valley, in Portugal, and its correlation to urban and industrial pollution. *Science against microbial pathogens: communicating current research and technological advances.*

Poulter, L. W., Seymour, G. J., Duke, O., Janossy, G., & Panayi, G. (1982). Immunohistological analysis of delayed-type hypersensitivity in man. *Cellular Immunology*, *74*(2), 358-369.

Prasad, M. P., Jayalakshmi, K., & Rindhe, G. G. (2012). Antibacterial activity of Ocimum species and their phytochemical and antioxidant potential. *International Journal of Microbiology Research*, *4*(8), 302.

Rafailidis, P. I., Bliziotis, I. A., & Falagas, M. E. (2010). Case–control studies reporting on risk factors for emergence of antimicrobial resistance: bias associated with the selection of the control group. *Microbial Drug Resistance*, *16*(4), 303-308.

Rahman, M. F., Siddiqui, M. K., & Jamil, K. (2001). Effects of Vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. *Human & Experimental Toxicology*, *20*(5), 243-249.

Rahmatollah, R., & Mahbobeh, R. (2010). Mineral contents of some plants used in Iran. *Pharmacognosy Research*, *2*(4), 267.

Ramadan, A., Soliman, G., Mahmoud, S. S., Nofal, S. M., & Abdel-Rahman, R. F. (2012). Evaluation of the safety and antioxidant activities of *Crocus sativus* and Propolis ethanolic extracts. *Journal of Saudi Chemical Society*, *16*(1), 13-21.

Raphael, I., Nalawade, S., Eagar, T. N., & Forsthuber, T. G. (2015). T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine*, *74*(1), 5-17.

Rasekh, H. R., Hosseinzadeh, L., Mehri, S., Kamli-Nejad, M., Aslani, M., & Tanbakoosazan, F. (2012). Safety assessment of *Ocimum basilicum* hydroalcoholic extract in wistar rats: acute and subchronic toxicity studies. *Iranian Journal of Basic Medical Sciences*, *15*(1), 645.

Rathnayaka, R. (2013). Antibacterial Activity of *Ocimum sanctum* extracts against four food-borne microbial pathogens. *Scholars Journal of Applied Medical Sciences*, *1*(6), 774-777.

Ray, W. A., Murray, K. T., Hall, K., Arbogast, P. G., & Stein, C. M. (2012). Azithromycin and the risk of cardiovascular death. *New England Journal of Medicine*, *366*(20), 1881-1890.

Rayman, M. P. (2000). The importance of selenium to human health. *The Lancet*, *356*(9225), 233-241.

Rigby, K. M., & DeLeo, F. R. (2012, March). Neutrophils in innate host defense against *Staphylococcus aureus* infections. *Seminars in Immunopathology*, *34*(2), 237-259.

Roig-Molina, E., Domenech, M., de Gracia Retamosa, M., Nácher-Vázquez, M., Rivas, L., Maestro, B., & Sanz, J. M. (2019). Widening the antimicrobial spectrum of esters of bicyclic amines: *In vitro* effect on gram-positive *Streptococcus pneumoniae* and gram-negative non-typeable *Haemophilus influenzae* biofilms. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1863*(1), 96-104.

Rooijakkers, S. H., van Kessel, K. P. & van Strijp, J. A. (2005). Staphylococcal innate immune evasion. *Trends in Microbiology,* 13:596–601

Rosato, A., Maggi, F., Cianfaglione, K., Conti, F., Ciaschetti, G., Rakotosaona, R., & Corbo, F. (2018). Chemical composition and antibacterial activity of seven uncommon essential oils. *Journal of Essential oil Research*, *30*(4), 233-243.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K., & Chinou, I. (2010). Chemical composition and antimicrobial activity of the essential oils of four Ocimum species growing in Tanzania. *Food Chemistry*, *119*(1), 311-316.

Ruppé, É., Woerther, P. L., & Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Annals of Intensive Care.* 5:61.

Sadashiv, P. S. (2010). Acute toxicity study of *Ocimum sanctum*. *International Research Journal of Pharmacy*, *1*(1), 409-413.

Sahu, M. S., Mali, P. Y., Waikar, S. B., & Rangari, V. D. (2010). Evaluation of immunomodulatory potential of ethanolic extract of *Roscoea procera* rhizomes in mice. *Journal of Pharmacy and Bioallied Sciences*, *2*(4), 346.

Sahu, M., Sahu, R., & Verma, A. (2013). Immunomodulatory activity of alcoholic extract of *Habenaria intermedia* in mice. *International Journal of Pharmacy and Pharmaceutical Science*, *5*(3), 406-409.

Sajjadi, S. E. (2006). Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. *Journal of Pharmaceutical Sciences*, *14*(3), 128-130.

Sakkas, H., & Papadopoulou, C. (2017). Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. *Journal of Microbiological Biotechnology, 28*(27), 3.

Sanni, S., Onyeyili, P. A., & Sanni, F. S. (2008). Phytochemical analysis, elemental determination and some *in vitro* antibacterial activity of *Ocimum basilicum* L. leaf extracts. *Research Journal of Phytochemistry*, *2*, 77-83.

Sappakul, P., Miltz, J., Sonneveld, K. & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural Food Chemistry,* 51:3197–3207.

Saris, N. E. L., Mervaala, E., Karppanen, H., Khawaja, J. A., & Lewenstam, A. (2000). Magnesium: an update on physiological, clinical and analytical aspects. *Clinica Chimica Acta*, *294*(1-2), 1-26.

Scarpa, E. S., & Ninfali, P. (2015). Phytochemicals as innovative therapeutic tools against cancer stem cells. *International Journal of Molecular Sciences*, *16*(7), 15727-15742.

Schmidt, J. M., Noletto, J. A., Vogler, B., & Setzer, W. N. (2007). Abaco bush medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. *Journal of herbs, spices & medicinal plants*, *12*(3), 43-65.

Segal, A. W. (2005). How neutrophils kill microbes. *Annual Review of Immunology*, *23*, 197-223.

Shah, A. S., Gunjal, M. A., & Juvekar, A. R. (2009). Immunomostimulatory activity of aqueous extract of *Azadirachta indica* flowers on specific and non-specific immune response. *Journal of Natural Remedies*, *9*(1), 35-42.

Shah, A., Niaz, A., Ullah, N., Rehman, A., Akhlaq, M., Zakir, M., & Suleman Khan, M. (2013). Comparative study of heavy metals in soil and selected medicinal plants. *Journal of Chemistry*, *2013*, Article ID 621265, 5 pages

Sharath, R., Harish, B. G., Krishna, V., Sathyanarayana, B. N., & Swamy, H. K. (2010). Wound healing and protease inhibition activity of Bacoside- A, isolated from *Bacopa monnieri* wettest. *Phytotherapy Research*, *24*(8), 1217-1222.

Sharififar, F., Pournourmohammadi, S., Rastegarianzadeh, R., Ranjbaran, O., & Purhemmaty, A. (2010). Immunomodulatory activity of aqueous extract of *Heracleum persicum* Desf. in mice. *Iranian Journal of Pharmaceutical Research*, *10*(4), 287-292.

Sharma, M., Pathak, S., & SrivaStava, P. (2013). Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. *Journal of Clinical and Diagnostic Research, 7*(10), 2173.

Sharma, P., Chambial, S., & Shukla, K. K. (2015). Lead and neurotoxicity. *Indian Journal of Clinical Biochemistry, 30*(1), 1–2.

Sharwan, G., Jain, P., Pandey, R., & Shukla, S. S. (2016). Toxicity and Safety Profiles of Methanolic Extract of *Pistacia integerrima* JL Stewart ex Brandis (PI) for Wistar Rats. *Journal of Pharmacopuncture*, *19*(3), 253.

Shibata, H., Kondo, K., Katsuyama, R., Kawazoe, K., Sato, Y., Murakami, K., & Higuti, T. (2005). Alkyl gallates, intensifiers of β-lactam susceptibility in methicillin-resistant *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy*, *49*(2), 549-555.

Shimoishi, K., Anraku, M., Kitamura, K., Tasaki, Y., Taguchi, K., Hashimoto, M., & Otagiri, M. (2007). An oral adsorbent, AST-120 protects against the progression of oxidative stress by reducing the accumulation of indoxyl sulfate in the systemic circulation in renal failure. *Pharmaceutical Research*, *24*(7), 1283-1289.

Shirazi, M. T., Gholami, H., Kavoosi, G., Rowshan, V., & Tafsiry, A. (2014). Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. *Food Science & Nutrition*, *2*(2), 146-155.

Shittu, A. O., Okon, K., Adesida, S., Oyedara, O., Witte, W., Strommenger, B., & Nübel, U. (2011). Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. *BioMed Central Microbiology*, *11*(1), 92.

Shore, A. C., Deasy, E. C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., ... & Coleman, D. C. (2011). Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *55*(8), 3765-3773.

Singh, A. R., Bajaj, V. K., Sekhawat, P. S., & Singh, K. (2013). Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum sanctum L. Journal of Natural Products and Plant Resources*, *3*(1), 51-8.

Singh, A., Bhat, T. K., & Sharma, O. P. (2011). Clinical Biochemistry of Hepatotoxicity. *Journal of Clinical Toxicology, 4*(001), 1-19.

Singh, R. K., & Watal, G. E. E. T. A. (2010). Antimicrobial potential of *Ficus bengalensis* aerial roots. *International Journal of Pharmaceutical and Biological Sciences*, *1*, 1-9.

Skalicka-Woźniak, K., Georgiev, M. I., & Orhan, I. E. (2017). Adulteration of herbal sexual enhancers and slimmers: The wish for better sexual well-being and perfect body can be risky. *Food and Chemical Toxicology*, *108*, 355-364.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science*, *4*(5), 200-222.

Sonmezer, M. C., Ertem, G., Erdinc, F. S., Kaya Kilic, E., Tulek, N., Adiloglu, A., & Hatipoglu, C. (2016). Evaluation of risk factors for antibiotic resistance in patients with nosocomial infections caused by *Pseudomonas aeruginosa. Canadian Journal of Infectious Diseases and Medical Microbiology*, *2016*, Article ID 1321487, 9 pages.

Spelman, K., Burns, J. J., Nichols, D., Winters, N., Ottersberg, S., & Tenborg, M. (2006). Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. *Alternative Medicine Review*, *11*(2), 128.

Spight, D., Zhao, B., Haas, M., Wert, S., Denenberg, A., & Shanley, T. P. (2005). Immunoregulatory effects of regulated, lung-targeted expression of IL-10 *in vivo*. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *288*(2), L251-L265.

Stanojkovic-Sebic, A., Pivic, R., Josic, D., Dinic, Z., & Stanojkovic, A. (2015). Heavy Metals Content in Selected Medicinal Plants Commonly Used as. *Tarım Bilimleri Dergisi*, *21*(3), 317-325.

Stapleton, P. D., Shah, S., Anderson, J. C., Hara, Y., Hamilton-Miller, J. M., & Taylor, P. W. (2004). Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. *International Journal of Antimicrobial Agents*, *23*(5), 462-467.

Stegger, Á., Andersen, P. S., Kearns, A., Pichon, B., Holmes, M. A., Edwards, G., & Larsen, A. R. (2012). Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either mecA or the new mecA homologue mecALGA251. *Clinical Microbiology and Infection*, *18*(4), 395-400.

Stray, F. (1998). The Natural Guide to medicinal herbs and plants, Tiger Books International London, pp: 12-16.

Stuffins, C. B. (1967). The determination of phosphate and calcium in feeding stuffs. *Analyst*, *92*(1091), 107-111.

Sudan, R., Bhagat, M., Gupta, S., Singh, J., & Koul, A. (2014). Iron (FeII) chelation, ferric reducing antioxidant power, and immune modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). *BioMedical Research International*, *2014*.

Sudha, P., Asdaq, S. M. B., & Sunil, S. (2010). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. *Indian Journal of Physiology and Pharmacology*, *54*(2), 133-140.

Sudhahar, V., Kumar, S. A., Varalakshmi, P., & Sujatha, V. (2008). Protective effect of lupeol and lupeol linoleate in hypercholesterolemia associated renal damage. *Molecular and Cellular Biochemistry*, *317*(1-2), 11.

Sui, Y., Li, S., Shi, P., Wu, Y., Li, Y., Chen, W., & Lin, X. (2016). Ethyl acetate extract from *Selaginella doederleinii* Hieron inhibits the growth of human lung cancer cells A549 via caspase-dependent apoptosis pathway. *Journal of Ethnopharmacology*, *190*, 261-271.

Sun, X. Y., Liu, M. Y., Zhong, C. Y., Zheng, G. L., Lv, M. Y., Jing, B. T., & Wang, X. (2018). Synthesis and Antibacterial Evaluation of 2-Ethyl-1-(4-substituted) phenyl-1 H-imidazole Derivatives as Open-Chain Analogues of 7-Alkoxyl-4, 5-dihydro-imidazo [1, 2-a] quinolines. *Journal of the Brazilian Chemical Society*, *29*(4), 701-707.

Symoens, J. (1977). Levamisole in the modulation of the immune response: the current experimental and clinical state. *Middle-East Journal of Scientific Research, 3(2), 105-108.*

Szeto, C. C., Gillespie, K. M., & Mathieson, P. W. (2000). Levamisole induces interleukin-18 and shifts type 1/type 2 cytokine balance. *Immunology*, *100*(2), 217.

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerging Infectious Diseases*, *18*(5), 741.

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, *140*(6), 805-820.

Talabi, J. Y., & Makanjuola, S. A. (2017). Proximate, Phytochemical, and *In Vitro* Antimicrobial Properties of Dried Leaves from *Ocimum gratissimum*. *Preventive Nutrition and Food Science*, *22*(3), 191.

Tavil, Y., Sen, N., Hızal, F., Abacı, A., & Cengel, A. (2007). Mean platelet volume in patients with metabolic syndrome and its relationship with coronary artery disease. *Thrombosis Research*, *120*(2), 245-250.

Teffo, L. S., Aderogba, M. A., & Eloff, J. N. (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. angustifolia leaf extracts. *South African Journal of Botany*, *76*(1), 25-29.

Thammavongsa, V., Kim, H. K., Missiakas, D., & Schneewind, O. (2015). Staphylococcal manipulation of host immune responses. *Nature Reviews. Microbiology*, *13*(9), 529.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In *Biotechnology of Isoprenoids* (pp. 63-106). Springer, Cham.

Tilwari, A., Shukla, N. P., & Pathirissery, U. D. (2011). Immunomodulatory activity of the aqueous extract of seeds of *Abrus precatorius* Linn (Jequirity) in mice. *Iranian Journal of Immunology*, *8*(2), 96.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

Togashi, N., Shiraishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H., & Inoue, Y. (2007). Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. *Molecules*, *12*(2), 139-148.

Tripathi, A. S., Chitra, V., Sheikh, N. W., Mohale, D. S., & Dewan, A. P. (2010). Immunomodulatory activity of the methanol extract of *Amorphophallus campanulatus* (Araceae) tuber. *Tropical Journal of Pharmaceutical Research*, *9*(5).

Tucci, S. A. (2010). Phytochemicals in the control of human appetite and body weight. *Pharmaceuticals*, *3*(3), 748-763.

Uddin, S. N., Akond, M. A., Mubassara, S., & Yesmin, M. N. (2008). Antioxidant and Antibacterial activities of *Trema cannabina*. *Middle-East Journal of Scientific Research*, *3*(2), 105-108.

Ugwu, M. N., Umar, I. A., Utu-Baku, A. B., Dasofunjo, K., Ukpanukpong, R. U., Yakubu, O. E., & Okafor, A. I. (2013). Antioxidant Status and Organ Function in Streptozotocin-Induced Diabetic Rats treated with Aqueous, Methanolic and Petroleum Ether Extracts of *Ocimum basilicum* leaf. *Journal of Applied Pharmaceutical Science*, 3(4), 75-79.

Ullah, R., Khader, J. A., Hussain, I., Talha, N. M. A., & Khan, N. (2012). Investigation of macro and micro-nutrients in selected medicinal plants. *African Journal of Pharmacy and Pharmacology*, *6*(25), 1829-1832.

Uma, M., Suresh, M., Thulasiraman, K., Lakshmidevi, E., & Kalaiselvi, P. (2013). Chronic toxicity studies of aqueous leaf extract of Indian traditional medicinal plant *Ocimum tenuiflorum* (Linn.) in rats. *European Journal of Experimental Biology*, *3*, 240-7.

Une, H. D., & Doshi, G. M. (2016). *Carissa congesta* (Wight) and *Benincasa hispida* (Thunb.) Cogn. as budding immunomodulatory agents. *Indian Journal of Experimental Biology, 54*(10), 650-658.

Vagdatli, E., Gounari, E., Lazaridou, E., Katsibourlia, E., Tsikopoulou, F., & Labrianou, I. (2010). Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia*, *14*(1), 28.

Vaghasiya, J., Datani, M., Nandkumar, K., Malaviya, S., & Jivani, N. (2010). Comparative evaluation of alcoholic and aqueous extracts of *Ocimum sanctum* for immunomodulatory activity. *International Journal of Pharmaceutical and Biological Research*, *1*(1), 25-29.

Van Andel, T. R., Croft, S., Van Loon, E. E., Quiroz, D., Towns, A. M., & Raes, N. (2015). Prioritizing West African medicinal plants for conservation and sustainable extraction studies based on market surveys and species distribution models. *Biological Conservation*, *181*, 173-181.

Venier, A. G., Leroyer, C., Slekovec, C., Talon, D., Bertrand, X., Parer, S., & Clair, B. (2014). Risk factors for *Pseudomonas aeruginosa* acquisition in intensive care units: a prospective multicentre study. *Journal of Hospital Infection*, *88*(2), 103-108.

Victor, D., Revathi, G., Sam, K., Abdi, H., Asad, R., & Andrew, K. (2013). Pattern of pathogens and their sensitivity isolated from surgical site infections at the Aga Khan University Hospital, Nairobi, Kenya. *Ethiopian Journal of Health Sciences*, *23*(2), 141-149.

Vidhani, S. I., Vyas, V. G., Parmar, H. J., Bhalani, V. M., Hassan, M. M., Gaber, A., & Golakiya, B. A. (2016). Evaluation of some chemical composition, minerals fatty acid profiles, antioxidant and antimicrobial activities of Tulsi (*Ocimum sanctum*) from India. *American Journal of Food Science and Technology*, *4*(2), 52-57.

Vimala, V., Mathew, R., Sankar, P. D., & Kalaivani, T. (2014). Phytochemical analysis in Ocimum accessions, *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(1), 555-557.

Vincent, J. L., Rello Condomines, J., Marshall, J., Silva, E., Anzueto, A., Martin, C. D., & Reinhart, K. (2009). The extended prevalence of infection in the ICU study: EPIC II. *Journal of the American Medical Association*, *302*(21), 0001-49.

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L., & McKenzie, K. K. (1987, January). Pathway to carrageenan-induced inflammation in the hind limb of the rat. In *Federation Proceedings* (Vol. 46, No. 1, pp. 118-126).

Vinothapooshan, G., & Sundar, K. (2011). Immunomodulatory activity of various extracts of *Adhatoda vasica* Linn. in experimental rats. *African Journal of Pharmacy and pharmacology*, *5*(3), 306-310.

Vlase, L., Benedec, D., Hanganu, D., Damian, G., Csillag, I., Sevastre, B., & Tilea, I. (2014). Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis, Ocimum basilicum* and *Teucrium chamaedrys*. *Molecules*, *19*(5), 5490-5507.

Waksman, B. H. (1979). Cellular hypersensitivity and immunity: conceptual changes in last decade. *Cellular Immunology*, *42*(1), 155-169.

Wang, J., Roderiquez, G., & Norcross, M. A. (2012). Control of adaptive immune responses by *Staphylococcus aureus* through IL-10, PD-L1, and TLR2. *Scientific Reports*, *2*, 606.

Wang, K., Ping, S., Huang, S., Hu, L., Xuan, H., Zhang, C., & Hu, F. (2013). Molecular mechanisms underlying the *in vitro* anti-inflammatory effects of a flavonoid-rich ethanol extract from Chinese propolis (poplar type). *Evidence-Based Complementary and Alternative Medicine*, *2013*. Article ID 127672, 11 pages.

Wang, Q., Zhang, Y., Yao, X., Xian, H., Liu, Y., Li, H., & Cao, B. (2016). Risk factors and clinical outcomes for carbapenem-resistant Enterobacteriaceae nosocomial infections. *European Journal of Clinical Microbiology & Infectious Diseases*, *35*(10), 1679-1689.

Wayne, P. A. (2015). CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. *CLSI Document M100-S25, Clinical and Laboratory Standards Institute*.

Weis, R., & Seebacher, W. (2009). New bicyclic amines: synthesis and SARs of their action against the causative organisms of malaria and sleeping sickness. *Current Medicinal Chemistry*, *16*(11), 1426-1441.

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., & Nouwen, J. L. (2005). The role of nasal carriage in *Staphylococcus aureus* infections. *The Lancet Infectious Diseases*, *5*(12), 751-762.

WHO, (2011). Report on the burden of endemic health care-associated infection worldwide. Retrieved from http://apps.who.int/iris/handle/10665/80135 on 21st June, 2017.

WHO, (2012). Prevention of hospital-acquired infections. Adapted from http://www.who.int/csr/resources/publications/whocdscsreph200212.pdf?ua=1 on 23rd June, 2017

Wintrobe, M. M. (2009). Wintrobe's clinical hematology (Vol. 1). Lippincott Williams & Wilkins.

Wonnenberg, B., Bischoff, M., Beisswenger, C., Dinh, T., Bals, R., Singh, B., & Tschernig, T. (2016). The role of IL-1β in *Pseudomonas aeruginosa* in lung infection. *Cell and Tissue Research*, *364*(2), 225-229.

Wood, J. H., Nthumba, P. M., Stepita-Poenaru, E., & Poenaru, D. (2012). Pediatric surgical site infection in the developing world: a Kenyan experience. *Pediatric Surgery International*, *28*(5), 523-527.

World Health Organization, WHO (2005). Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, Switzerland.

World Health Organization, WHO (2006). Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues, World Health Organization, Geneva, Switzerland.

World Health Organization, WHO (2011). African Partnerships for Patient Safety. Available from: http://www.who.int/patientsafety/implementation/apps/en/index.html (Accessed on 26th June 2017).

Xiong, L., Peng, C., & Zhou, M. (2013). "Chemical composition and antibacterial activity of essential oils from different parts of *Leonurus japonicus* houtt," *Molecules*, vol. 18, no. 1, pp. 963–973.

Yadav, M. K., Chae, S. W., Im, G. J., Chung, J. W., & Song, J. J. (2015). Eugenol: a phyto-compound effective against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* clinical strain biofilms. *PLoS One*, *10*(3), e0119564.

Yahya, N. Z. (2011). *In vitro* and *In vivo* evaluation of antimicrobial effect of ethanolic leaf extracts of *Ocimum basilicum* against *Staphylococcus aureus*. *Journal of Veterinary Science,* 4(2).

Yakubu, M. T., Bilbis, L. S., Lawal, M., & Akanji, M. A. (2003). Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biokemistri*, *15*(2), 50-56.

Yamaguchi, T. (2019). Antibacterial properties of nootkatone against Gram-positive bacteria. *Natural Product Communications*, *14*(6), 1934578X19859999.

Yamani, H. A., Pang, E. C., Mantri, N., & Deighton, M. A. (2016). Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. *Frontiers in Microbiology*, *7*, 681.

Yang, S. J., Kreiswirth, B. N., Sakoulas, G., Yeaman, M. R., Xiong, Y. Q., Sawa, A., & Bayer, A. S. (2009). Enhanced expression of dltABCD is associated with the development of daptomycin non-susceptibility in a clinical endocarditis isolate of *Staphylococcus aureus*. *The Journal of Infectious Diseases*, *200*(12), 1916-1920.

Yen, H., Karino, M., & Tobe, T. (2016). Modulation of the inflammasome signaling pathway by enteropathogenic and enterohemorrhagic *Escherichia coli*. *Frontiers in Cellular and Infection Microbiology*, *6*, 89.

Yokoe, I., Azuma, K., Hata, K., Mukaiyama, T., Goto, T., Tsuka, T. & Minami, S. (2015). Clinical systemic lupeol administration for canine oral malignant melanoma. *Molecular and Clinical Oncology*, *3*(1), 89-92.

Zhang, H. Y., Gao, Y., & Lai, P. X. (2017). Chemical composition, antioxidant, antimicrobial and cytotoxic activities of essential oil from *Premna microphylla Turczaninow*. *Molecules*, *22*(3), 381.

Zhang, S., Cui, Y. L., Diao, M. Y., Chen, D. C., & Lin, Z. F. (2015). Use of platelet indices for determining illness severity and predicting prognosis in critically ill patients. *Chinese Medical Journal*, *128*(15), 2012.

Zhao, W. H., & Hu, Z. Q. (2010). β-lactamases identified in clinical isolates of *Pseudomonas aeruginosa*. *Critical Reviews in Microbiology*, *36*(3), 245-258.

Zheng, C. J., Yoo, J. S., Lee, T. G., Cho, H. Y., Kim, Y. H., & Kim, W. G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS letters*, *579*(23), 5157-5162.

Zhou, R., Yazdi, A. S., Menu, P., & Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature*, *469*(7329), 221-225.

Zielinski, C. E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., & Sallusto, F. (2012). Pathogen-induced human T^ sub H^ 17 cells produce IFN-[gamma] or IL-10 and are regulated by IL-1 [Beta]. *Nature*, *484*(7395), 514.

APPENDICES

Appendix I: Washing Red Blood Cells

i. The blood was centrifuged at 5000 rpms.

ii. With the aid of a clean pipette, the supernatant was pipetted out and discarded.

iii. The RRBCs were mixed with PBS in a ratio of 1:1 and centrifuged for 30 seconds.

iv. With the aid of a clean pipette, the supernatant was discarded.

v. Steps iii and iv were repeated thrice.

vi. RRBCs were mixed with PBS in a ratio of 1:1 and run on an auto-agglutination card (upon agglutination, the washing procedure is repeated).

AppendiX II: Effect of *O. basilicum* Ethyl acetate Leaf Extract on Mean Foot Paw Edema in DTH Models

Group	Treatment	Dose	Mean Foot Paw Edema in mm							
			Hour 2	Hour 4	Hour 6	Hour 8	Hour 12	Hour 24	Hour 36	Hour 48
I	4% DMSO	10 ml/kgbwt	0.271 ± 0.215^{a}	0.279 ± 0.022^{b}	0.358 ± 0.031^{c}	0.369 ± 0.024^{a}	0.304 ± 0.054^{a}	0.2730 ± 0.031^{a}	0.214 ± 0.050^{a}	0.149 ± 0.038^{a}
II	Cyclophosphamide	20 mg/kgbwt	0.271 ± 0.020^{a}	0.369 ± 0.053^{ab}	0.345 ± 0.559^{c}	0.350 ± 0.029^{a}	0.337 ± 0.026^{a}	0.242 ± 0.046^{a}	0.192 ± 0.059^{a}	0.121 ± 0.017^{a}
III	Levamisole	50 mg/kgbwt	0.316 ± 0.082^{a}	0.381 ± 0.053^{a}	0.471 ± 0.060^{ab}	0.381 ± 0.052^{a}	0.345 ± 0.030^{a}	0.211 ± 0.025^{a}	0.156 ± 0.021^{a}	0.079 ± 0.050^{b}
IV	O. B Extract	100 mg/kgbwt	0.294 ± 0.061^{a}	0.443 ± 0.072^{a}	0.432 ± 0.051^{abc}	0.348 ± 0.036^{a}	0.284 ± 0.054^{a}	0.214 ± 0.041^{a}	0.208 ± 0.049^{a}	0.169 ± 0.059^{a}
V	O. B Extract	200 mg/kgbwt	0.306 ± 0.022^{a}	0.376 ± 0.021^{a}	0.382 ± 0.022^{bc}	0.371 ± 0.046^{a}	0.303 ± 0.069^{a}	0.255 ± 0.018^{a}	0.165 ± 0.052^{a}	0.099 ± 0.027^{a}
VI	O. B Extract	300 mg/kgbwt	0.273 ± 0.057^{a}	0.415 ± 0.048^{a}	0.477 ± 0.056^{a}	0.396 ± 0.063^{a}	0.337 ± 0.033^{a}	0.271 ± 0.087^{a}	0.203 ± 0.068^{a}	0.094 ± 0.047^{a}

Values are expressed as Mean ± S.D for n=5. Values with the same superscript letter in the same column are not significantly different by One Way ANOVA followed by Tukey's post hoc test for pairwise comparison (P ≤ 0.05).

Appendix III: Statistical Analyses for Antimicrobial Assays

Grouping Information Using the Tukey Method and 95% Confidence Interval (Means that do not share a letter are significantly different)

MRSA ATCC 43300

Factor	N	Mean	Grouping
Genta	3	32.33	A
OB100	3	25.00	B
OB75	3	23.333	B
OB50	3	22.000	B
OB25	3	18.000	C
DMSO	3	6.000	D

***S. aureus ATCC* 25923**

Factor	N	Mean	Grouping
Genta	3	33.000	A
Neomycin	3	27.667	B
100.00%	3	24.667	C
75.00%	3	23.000	C D
50.00%	3	21.667	D
25.00%	3	18.000	E
DMSO	3	6.000	F

***S. aureus* (isolate)**

Factor	N	Mean	Grouping
Genta	3	29.000	A
100.00%	3	24.000	B
75.00%	3	21.667	B C
50.00%	3	20.333	C
25.00%	3	17.333	D
Neomycin	3	13.333	E
DMSO	3	6.000	F

E. coli ATCC 29211

Factor	N	Mean	Grouping
Genta	3	32.000	A
Neomycin	3	31.333	A
100.00%	3	26.000	B
75.00%	3	24.667	B
50.00%	3	22.67	B C
25.00%	3	20.000	C
DMSO	3	6.000	D

E. coli (isolate)

Factor	N	Mean	Grouping
Genta	3	27.000	A
100.00%	3	24.667	A B
75.00%	3	22.667	B C
50.00%	3	21.333	C D
25.00%	3	18.667	D
Neomycin	3	15.333	E
DMSO	3	6.000	F

P. aeruginosa ATCC 27853

Factor	N	Mean	Grouping
Genta	3	31.333	A
100.00%	3	27.00	B
Neomycin	3	27.000	B
75.00%	3	23.333	B C
50.00%	3	22.33	C
25.00%	3	19.667	C
DMSO	3	6.000	D

P. aeruginosa (isolate)

Factor	N	Mean	Grouping
Genta	3	28.667	A
100.00%	3	26.667	A
75.00%	3	21.667	B
50.00%	3	21.333	B
25.00%	3	18.667	B
Neomycin	3	14.33	C
DMSO	3	6.000	D

Two Sample T – Tests for *S. aureus*

Two-sample T for *S.aureus* (ATCC)Genta vs S. a (isolate)Genta

	N	Mean	StDev	SE Mean
S.a (s)Genta	3	33.00	1.00	0.58
S. a (i)Genta	3	29.00	1.00	0.58

T-Test of difference = 0 (vs ≠): T-Value = 4.90 P-Value = 0.008 DF = 4

Two-sample T for *S. aureus* (isolate)Neo vs S. a (ATCC)Neo

	N	Mean	StDev	SE Mean
S. a (i)Neo	3	13.333	0.577	0.33
S. a (s)Neo	3	27.667	0.577	0.33

T-Test of difference = 0 (vs ≠): T-Value = -30.41 P-Value = 0.000 DF = 4

Two-sample T for S. a (i)100% vs S. a (s)100%

```
               N    Mean  StDev  SE Mean
S. a (i)100%  3   24.00   1.00     0.58
S. a (s)100%  3  24.667  0.577     0.33
```

T-Test of difference = 0 (vs ≠): T-Value = -1.00 P-Value = 0.391 DF = 3

Two-sample T for S. a (i)75% vs S.a (s)75%

```
              N   Mean  StDev  SE Mean
S. a (i)75%  3  21.67   1.53     0.88
S.a (s)75%   3  23.00   1.00     0.58
```

T-Test of difference = 0 (vs ≠): T-Value = -1.26 P-Value = 0.295 DF = 3

Two-sample T for S.a (i)50% vs S.a (s)50%

```
             N    Mean  StDev  SE Mean
S.a (i)50%  3   20.33   1.53     0.88
S.a (s)50%  3  21.667  0.577     0.33
```

T-Test of difference = 0 (vs ≠): T-Value = -1.41 P-Value = 0.293 DF = 2

Two-sample T for S. a (i)25% vs S.a (s)25%

```
              N    Mean  StDev  SE Mean
S. a (i)25%  3  17.333  0.577     0.33
S.a (s)25%   3   18.00   1.00     0.58
```

T-Test of difference = 0 (vs ≠): T-Value = -1.00 P-Value = 0.391 DF = 3

Two Sample T Tests for *E. coli*

Two-sample T for E. c (s) Genta vs E. c (i)Genta

```
                N   Mean  StDev  SE
Mean
E. c (s) Genta  3  32.00   1.00
0.58
E. c (i)Genta   3  27.00   1.00
0.58
```

T-Test of difference = 0 (vs ≠): T-Value = 6.12 P-Value = 0.004 DF = 4

Two-sample T for E. c (s)Neo vs E. c (i)Neo

```
              N    Mean  StDev  SE Mean
E. c (s)Neo  3  31.333  0.577     0.33
E. c (i)Neo  3  15.333  0.577     0.33
```

T-Test of difference = 0 (vs ≠): T-Value = 33.94 P-Value = 0.000 DF = 4

Two-sample T for E. c (s)100% vs E. c (i)100%

```
               N    Mean  StDev  SE Mean
E. c (s)100%  3   26.00   1.00     0.58
E. c (i)100%  3  24.667  0.577     0.33
```

T-Test of difference = 0 (vs ≠): T-Value = 2.00 P-Value = 0.139 DF = 3

Two-sample T for E. c (s)75% vs E.c (i)75%

```
              N   Mean  StDev  SE Mean
E. c (s)75%  3  24.67   1.53     0.88
E.c (i)75%   3  22.67   1.53     0.88
```

T-Test of difference = 0 (vs ≠): T-Value = 1.60 P-Value = 0.184 DF = 4

Two-sample T for E. c (s)50% vs E. c (i)50%

```
              N   Mean  StDev  SE Mean
E. c (s)50%  3  22.67   2.52      1.5
E. c (i)50%  3  21.33   1.53     0.88
```

T-Test of difference = 0 (vs ≠): T-Value = 0.78 P-Value = 0.490 DF = 3

Two-sample T for E.c (s) 25% vs E. c (i)25%

```
              N   Mean  StDev  SE Mean
E.c (s) 25%  3  20.00   1.00     0.58
E. c (i)25%  3  18.67   1.53     0.88
```

T-Test of difference = 0 (vs ≠): T-Value = 1.26 P-Value = 0.295 DF = 3

T Tests for *P. aeruginosa*

Two-sample T for P. a (s)Genta vs P.a (i)Genta

	N	Mean	StDev	SE Mean
P. a (s)Genta	3	31.33	1.53	0.88
P.a (i)Genta	3	28.67	1.53	0.88

T-Test of difference = 0 (vs ≠): T-Value = 2.14 P-Value = 0.099 DF = 4

Two-sample T for P. a (s)Neo vs P.a (i)Neo

	N	Mean	StDev	SE Mean
P. a (s)Neo	3	27.00	1.00	0.58
P.a (i)Neo	3	14.33	2.08	1.2

T-Test of difference = 0 (vs ≠): T-Value = 9.50 P-Value = 0.011 DF = 2

Two-sample T for P.a (s)100% vs P.a (i)100%

	N	Mean	StDev	SE Mean
P.a (s)100%	3	27.00	2.00	1.2
P.a (i)100%	3	26.67	1.53	0.88

T-Test of difference = 0 (vs ≠): T-Value = 0.23 P-Value = 0.833 DF = 3

Two-sample T for P.a (s)75% vs P.a (i)75%

	N	Mean	StDev	SE Mean
P.a (s)75%	3	23.333	0.577	0.33
P.a (i)75%	3	21.67	1.15	0.67

T-Test of difference = 0 (vs ≠): T-Value = 2.24 P-Value = 0.155 DF = 2

Two-sample T for P. a(s)50% vs P.a (i)50%

	N	Mean	StDev	SE Mean
P. a(s)50%	3	22.33	2.52	1.5
P.a (i)50%	3	21.33	1.53	0.88

T-Test of difference = 0 (vs ≠): T-Value = 0.59 P-Value = 0.598 DF = 3

Two-sample T for P.a (s)25% vs P.a (i)25%

	N	Mean	StDev	SE Mean
P.a (s)25%	3	19.667	0.577	0.33
P.a (i)25%	3	18.67	1.53	0.88

T-Test of difference = 0 (vs ≠): T-Value = 1.06 P-Value = 0.400 DF = 2

Statistical Analyses for Immune Modulation

Grouping Information Using the Tukey Method and 95% Confidence Interval for the Effects of the Extract on Cyclophosphamide Induced Myelosuppression

Hemoglobin Day 0

Factor	N	Mean	Grouping
DOSE 3	5	11.740	A
CYP	5	11.684	A
DOSE 1	5	11.658	A
DOSE 2	5	11.436	A
CTRL	5	11.356	A
LEV	5	11.188	A

Hemoglobin Day 14

Factor	N	Mean	Grouping
DOSE 3	5	12.688	A
DOSE 2	5	12.068	A B
LEV	5	11.422	A B
DOSE 1	5	11.284	B
CTRL	5	11.182	B
CYP	5	6.612	C

WBC Counts Day 0

Factor	N	Mean	Grouping
LEV	5	9.736	A
CTRL	5	9.698	A
DOSE 1	5	9.306	A
DOSE 2	5	9.232	A
DOSE 3	5	8.936	A
CYP	5	8.884	A

WBC Counts Day 14

Factor	N	Mean	Grouping
LEV	5	10.110	A
Cont	5	9.622	A
DOSE 3	5	8.002	B
DOSE 2	5	7.350	B
DOSE 1	5	6.852	B
CP	5	4.378	C

RBC Day 0

Factor	N	Mean	Grouping
Cont	5	7.836	A
DOSE 3	5	7.760	A
DOSE 1	5	7.712	A
CP	5	7.712	A
LEV	5	7.698	A
DOSE 2	5	7.614	A

RBC Day 14

Factor	N	Mean	Grouping
DOSE 3	5	9.182	A
DOSE 2	5	8.324	A B
Cont	5	8.214	A B
LEV	5	8.074	A B
DOSE 1	5	7.398	B C
CP	5	6.304	C

2 Sample T test For Blood Cell Counts on Day 0 and Day 14

Hemoglobin (Control)

Two-sample T for Cont d0 vs Cont d14

	N	Mean	StDev	SE Mean
Cont d0	5	11.356	0.793	0.35
Cont d14	5	11.182	0.574	0.26

T-Test of difference = 0 (vs ≠): T-Value = 0.40 P-Value = 0.703 DF = 7

Hemoglobin (cyclophosphamide)

Two-sample T for CP d0 vs CP d14

	N	Mean	StDev	SE Mean
CP d0	5	11.684	0.517	0.23
CP d14	5	6.612	0.430	0.19

T-Test of difference = 0 (vs ≠): T-Value = 16.86 P-Value = 0.000 DF = 7

Hemoglobin (Levamisole)

Two-sample T for LEV d0 vs LEV d14

	N	Mean	StDev	SE Mean
LEV d0	5	11.188	0.652	0.29
LEV d14	5	11.422	0.774	0.35

T-Test of difference = 0 (vs ≠): T-Value = -0.52 P-Value = 0.621 DF = 7

Hemoglobin (100mg/kgbw)

Two-sample T for DOSE 1 d0 vs DOSE 1 d14

	N	Mean	StDev	SE Mean
DOSE 1 d0	5	11.658	0.663	0.30
DOSE 1 d14	5	11.284	0.876	0.39

T-Test of difference = 0 (vs ≠): T-Value = 0.76 P-Value = 0.471 DF = 7

Hemoglobin (200mg/kgbw)

Two-sample T for DOSE 2 d0 vs DOSE 2 d14

	N	Mean	StDev	SE Mean
DOSE 2 d0	5	11.436	0.843	0.38
DOSE 2 d14	5	12.068	0.512	0.23

T-Test of difference = 0 (vs ≠): T-Value = -1.43 P-Value = 0.202 DF = 6

Hemoglobin (300mg/kgbw)

Two-sample T for DOSE 3 d0 vs DOSE 3 d14

	N	Mean	StDev	SE Mean
DOSE 3 d0	5	11.740	0.582	0.26
DOSE 3 d14	5	12.688	0.831	0.37

T-Test of difference = 0 (vs ≠): T-Value = -2.09 P-Value = 0.075 DF = 7

WBC (Control)

Two-sample T for Cont do vs Cont d14

	N	Mean	StDev	SE Mean
Cont do	5	9.698	0.623	0.28
Cont d14	5	9.622	0.992	0.44

T-Test of difference = 0 (vs ≠): T-Value = 0.15 P-Value = 0.889 DF = 6

WBC (Cyclophosphamide)

Two-sample T for CP d0 vs CP d14

	N	Mean	StDev	SE Mean
CP d0	5	8.884	0.534	0.24
CP d14	5	4.378	0.691	0.31

T-Test of difference = 0 (vs ≠): T-Value = 11.54 P-Value = 0.000 DF = 7

WBC (Levamisole)

Two-sample T for LEV d0 vs LEV d14

	N	Mean	StDev	SE Mean
LEV d0	5	9.736	0.858	0.38
LEV d14	5	10.110	0.652	0.29

T-Test of difference = 0 (vs ≠): T-Value = -0.78 P-Value = 0.463 DF = 7

WBC (100mg/kgbw)

Two-sample T for DOSE 1 d0 vs DOSE 1 d14

	N	Mean	StDev	SE Mean
DOSE 1 d0	5	9.306	0.689	0.31
DOSE 1 d14	5	6.852	0.358	0.16

T-Test of difference = 0 (vs ≠): T-Value = 7.06 P-Value = 0.000 DF = 6

WBC (200mg/kgbw)

Two-sample T for DOSE 2 d0 vs DOSE 2 d14

	N	Mean	StDev	SE Mean
DOSE 2 d0	5	9.232	0.676	0.30
DOSE 2 d14	5	7.350	0.827	0.37

T-Test of difference = 0 (vs ≠): T-Value = 3.94 P-Value = 0.006 DF = 7

WBC (300mg/kgbw)

Two-sample T for DOSE 3 d0 vs DOSE 3 d14

	N	Mean	StDev	SE Mean
DOSE 3 d0	5	8.936	0.955	0.43
DOSE 3 d14	5	8.002	0.356	0.16

T-Test of difference = 0 (vs ≠): T-Value = 2.05 P-Value = 0.096 DF = 5

Tukey Pairwise Comparisons for different Concentrations in Mean Hemagglutinin titer tests

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
300 mg/kgbw	5	12.400	A
LEV	5	11.600	A
200 mg/kgbw	5	9.600	B
100 mg/kgbw	5	8.800	B
Control	5	8.400	B
CYP	5	5.600	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons for Nylon Fiber Treated and Untreated Blood Neutrophil Indexes

Grouping Information Using the Tukey's and 95% Confidence for Untreated Bld

Factor	N	Mean	Grouping
LEV	5	567.4	A
DOSE 3	5	489.7	B
DOSE 2	5	396.34	C
DOSE 1	5	348.6	D
CTRL	5	285.84	E
CYP	5	259.85	E

Means that do not share a letter are significantly different

Grouping Information Using the Tukey Method and 95% Confidence for Treated Bld

Factor	N	Mean	Grouping
LEV	5	491.2	A
DOSE 3	5	435.6	B
DOSE 2	5	369.50	C
DOSE 1	5	313.52	D
CTRL	5	241.30	E
CYP	5	234.5	E

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence for Neutrophil Adhesion

Factor	N	Mean	Grouping
CTRL	5	15.27	A
LEV	5	13.38	A
DOSE 3	5	11.084	A
DOSE 1	5	10.58	A
CYP	5	9.90	A
DOSE 2	5	6.80	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Delayed Type Hypersensitivity at Different Hours

HOUR 2

Factor	N	Mean	Grouping
CTRL	5	0.3163	A
DOSE 2	5	0.30598	A
DOSE 1	5	0.2940	A
CYCL	5	0.2733	A
DOSE 3	5	0.27100	A
LEV	5	0.27083	A

HOUR 4

Factor	N	Mean	Grouping
DOSE 1	5	0.4425	A
CYCL	5	0.4150	A
CTRL	5	0.3810	A
DOSE 2	5	0.37556	A
DOSE 3	5	0.3687	A B
LEV	5	0.27980	B

HOUR 6

Factor	N	Mean	Grouping
CYCL	5	0.4772	A
CTRL	5	0.4705	A B
DOSE 1	5	0.4320	A B C
DOSE 2	5	0.3815	B C
LEV	5	0.3583	C
DOSE 3	5	0.3445	C

HOUR 8

Factor	N	Mean	Grouping
CYCL	5	0.3956	A
CTRL	5	0.3813	A
DOSE 2	5	0.3712	A
LEV	5	0.3688	A
DOSE 3	5	0.3503	A
DOSE 1	5	0.3477	A

HOUR 12

Factor	N	Mean	Grouping
CTRL	5	0.3450	A
CYCL	5	0.3369	A
DOSE 3	5	0.3367	A
LEV	5	0.3041	A
DOSE 2	5	0.3026	A
DOSE 1	5	0.2840	A

HOUR 24

Factor	N	Mean	Grouping
LEV	5	0.2730	A
CYCL	5	0.2706	A
DOSE 2	5	0.25458	A
DOSE 3	5	0.2423	A
DOSE 1	5	0.2139	A
CTRL	5	0.2113	A

HOUR 36

Factor	N	Mean	Grouping
LEV	5	0.2135	A
DOSE 1	5	0.2082	A
CYCL	5	0.2029	A
DOSE 3	5	0.1916	A
DOSE 2	5	0.1652	A
CTRL	5	0.15578	A

HOUR 48

Factor	N	Mean	Grouping
DOSE 1	5	0.1694	A
LEV	5	0.1492	A B
DOSE 3	5	0.12149	A B
DOSE 2	5	0.0996	A B
CYCL	5	0.0940	A B
CTRL	5	0.0787	B

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on White Blood Cell Indices and Platelet Indices in DTH Models

WBC Counts

Factor	N	Mean	Grouping
LEV	5	11.980	A
DOSE 3	5	9.720	A B
DOSE 2	5	8.160	B C
DOSE 1	5	7.500	B C
Cont	5	7.340	C
CP	5	6.480	C

Lymphocyte Counts

Factor	N	Mean	Grouping
LEV	5	87.14	A
DOSE 3	5	81.86	A B
DOSE 2	5	81.74	A B
Cont	5	78.10	A B
DOSE 1	5	77.78	A B
CP	5	74.76	B

Granulocytes

Factor	N	Mean	Grouping
LEV	5	9.740	A

Platelets

Factor	N	Mean	Grouping
DOSE 3	5	317.6	A

Factor	N	Mean	Grouping
DOSE 3	5	8.160	A B
DOSE 2	5	7.780	A B
DOSE 1	5	7.680	A B
Cont	5	7.460	B
CP	5	6.920	B

Factor	N	Mean	Grouping
DOSE 2	5	300.0	A
DOSE 1	5	276.6	A B
LEV	5	267.2	A B
Cont	5	229.6	A B
CP	5	201.4	B

Mean Platelet Volume

Factor	N	Mean	Grouping
DOSE 3	5	6.940	A
LEV	5	6.620	A
DOSE 2	5	6.360	A B
DOSE 1	5	6.260	A B
Cont	5	6.040	A B
CP	5	5.540	B

Platelet Distribution Width

Factor	N	Mean	Grouping
LEV	5	41.26	A
DOSE 3	5	40.820	A
DOSE 2	5	39.120	A B
DOSE 1	5	38.880	A B
Cont	5	38.560	A B
CP	5	37.720	B

Plateletcrit

Factor	N	Mean	Grouping
DOSE 1	5	0.1800	A
DOSE 3	5	0.1780	A
DOSE 2	5	0.1680	A B
Cont	5	0.12600	A B
LEV	5	0.1240	A B
CP	5	0.0960	B

P-LCR

Factor	N	Mean	Grouping
DOSE 2	5	5.660	A
DOSE 3	5	4.980	A
DOSE 1	5	4.460	A
Cont	5	4.140	A
LEV	5	3.920	A
CP	5	3.640	A

Statistical Analyses for Safety Evaluation

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Body Weights

Body Weights (1000mg/kgbw)

Factor	N	Mean	Grouping
Week 4	5	32.040	A
Week 3	5	30.60	A B
Week 2	5	29.22	A B
Week 1	5	26.780	B C
Acclima	5	24.16	C

Body Weights (548mg/kgbw)

Factor	N	Mean	Grouping
Week 4_1	5	33.080	A
Week 3_1	5	30.980	A B
Week 2_1	5	29.540	A B C
Week 1_1	5	26.680	B C
Acclima_1	5	25.12	C

Body Weights (300mg/kgbw)

Factor	N	Mean	Grouping
Week 4_1	5	31.58	A
Week 3_1	5	30.84	A B
Week 2_1	5	28.62	A B C
Week 1_1	5	26.06	B C
Acclima_1	5	24.960	C

Control group

Factor	N	Mean	Grouping
Week 4_1	5	32.34	A
Week 3_1	5	30.47	A B
Week 2_1	5	27.82	A B C
Week 1_1	5	24.48	B C
Acclima_1	5	23.38	C

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Organ Weights

Heart

Factor	N	Mean	Grouping
Dose 2	5	0.1260	A
Control	5	0.11000	A
Dose 1	5	0.10200	A

Brain

Factor	N	Mean	Grouping
Control	5	0.4340	A
Dose 1	5	0.3920	A
Dose 2	5	0.3860	A

Factor	N	Mean	Grouping
Dose 3	5	0.0980	A

Factor	N	Mean	Grouping
Dose 3	5	0.37000	A

Lungs

Factor	N	Mean	Grouping
Dose 1	5	0.3160	A
Dose 2	5	0.2800	A
Control	5	0.2340	A
Dose 3	5	0.2300	A

Testes

Factor	N	Mean	Grouping
Control	5	0.2400	A
Dose 3	5	0.2120	A
Dose 2	5	0.2120	A
Dose 1	5	0.2060	A

Kidney

Factor	N	Mean	Grouping
Dose 2	5	0.5100	A
Control	5	0.5060	A
Dose 3	5	0.4800	A
Dose 1	5	0.4520	A

Liver

Factor	N	Mean	Grouping
Dose 2	5	1.8340	A
Control	5	1.828	A
Dose 1	5	1.736	A
Dose 3	5	1.554	A

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Organ Indexes

Heart

Factor	N	Mean	Grouping
Dose 2	5	0.003835	A
Control	5	0.003389	A
Dose 1	5	0.003221	A
Dose 3	5	0.003053	A

Brain

Factor	N	Mean	Grouping
Control	5	0.013458	A
Dose 1	5	0.012407	A
Dose 2	5	0.011702	A
Dose 3	5	0.011561	A

Lungs

Factor	N	Mean	Grouping
Dose 1	5	0.01009	A
Dose 2	5	0.008462	A
Control	5	0.007246	A
Dose 3	5	0.007163	A

Testes

Factor	N	Mean	Grouping
Control	5	0.007458	A
Dose 3	5	0.00663	A
Dose 1	5	0.00659	A
Dose 2	5	0.006358	A

Kidney

Factor	N	Mean	Grouping
Control	5	0.015612	A
Dose 2	5	0.015398	A
Dose 3	5	0.014975	A
Dose 1	5	0.01436	A

Liver

Factor	N	Mean	Grouping
Control	5	0.05649	A
Dose 2	5	0.05547	A
Dose 1	5	0.05500	A
Dose 3	5	0.04821	A

Spleen

Factor	N	Mean	Grouping
Control	5	0.00855	A
Dose 1	5	0.00805	A
Dose 3	5	0.007924	A
Dose 2	5	0.007623	A

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Red Blood Cell Indices

Red Blood Cells

Factor	N	Mean	Grouping
Dose 3	5	8.0440	A
Control	5	7.778	A

Hemoglobin

Factor	N	Mean	Grouping
Dose 3	5	11.260	A
Control	5	11.160	A

Factor	N	Mean	Grouping
Dose 2	5	7.542	A
Dose 1	5	7.364	A

Factor	N	Mean	Grouping
Dose 2	5	10.420	A
Dose 1	5	9.960	A

MCH

Factor	N	Mean	Grouping
Control	5	14.380	A
Dose 3	5	14.000	A
Dose 2	5	13.900	A
Dose 1	5	13.540	A

MCV

Factor	N	Mean	Grouping
Dose 2	5	55.40	A
Control	5	55.10	A
Dose 3	5	54.16	A
Dose 1	5	51.00	A

MCHC

Factor	N	Mean	Grouping
Dose 1	5	26.600	A
Control	5	26.000	A
Dose 3	5	25.920	A
Dose 2	5	25.200	A

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on White Blood Cell Indices

White Blood Cells

Factor	N	Mean	Grouping
Control	5	10.26	A
Dose 1	5	9.66	A
Dose 3	5	9.18	A
Dose 2	5	8.34	A

Lymphocytes

Factor	N	Mean	Grouping
Control	5	80.12	A
Dose 3	5	78.88	A B
Dose 1	5	76.82	A B
Dose 2	5	70.28	B

Monocytes

Factor	N	Mean	Grouping
Dose 2	5	8.940	A
Dose 1	5	7.720	A
Dose 3	5	6.540	A
Control	5	6.440	A

Granulocytes

Factor	N	Mean	Grouping
Dose 2	5	20.78	A
Dose 1	5	15.46	A
Dose 3	5	13.96	A
Control	5	13.44	A

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Platelet Indices

Platelets

Factor	N	Mean	Grouping
Control	5	717.2	A
Dose 2	5	643.4	A
Dose 3	5	604	A
Dose 1	5	449.8	A

MPV

Factor	N	Mean	Grouping
Dose 1	5	4.780	A
Control	5	4.580	A
Dose 2	5	4.380	A
Dose 3	5	4.100	A

PDW

Factor	N	Mean	Grouping
Dose 2	5	16.980	A
Control	5	16.880	A
Dose 3	5	16.860	A
Dose 1	5	16.680	A

PCT

Factor	N	Mean	Grouping
Control	5	0.3280	A
Dose 2	5	0.2800	A
Dose 3	5	0.2580	A
Dose 1	5	0.2060	A

Tukey Pairwise Comparisons and Grouping Information on the Effects of Varying Doses of *O. basilicum* Extract on Biochemical Indices

ALT

Factor	N	Mean	Grouping
Dose 3	5	66.98	A
Dose 2	5	64.10	A
Dose 1	5	62.78	A
Control	5	62.120	A

AST

Factor	N	Mean	Grouping
Dose 3	5	198.64	A
Dose 2	5	183.02	A
Control	5	180.65	A
Dose 1	5	179.88	A

ALP

Factor	N	Mean	Grouping
Dose 3	5	16.40	A
Dose 2	5	15.000	A
Dose 1	5	13.00	A
Control	5	12.00	A

ALB

Factor	N	Mean	Grouping
Dose 3	5	18.44	A
Control	5	15.56	A
Dose 1	5	14.30	A
Dose 2	5	13.22	A

T-BIL

Factor	N	Mean	Grouping
Control	5	10.840	A
Dose 1	5	8.54	A
Dose 3	5	8.28	A
Dose 2	5	7.240	A

D-BIL

Factor	N	Mean	Grouping
Control	5	6.100	A
Dose 1	5	4.600	A
Dose 3	5	4.520	A
Dose 2	5	4.160	A

TP

Factor	N	Mean	Grouping
Control	5	42.50	A
Dose 3	5	42.10	A
Dose 1	5	41.860	A
Dose 2	5	39.400	A

TG

Factor	N	Mean	Grouping
Control	5	1.300	A
Dose 3	5	1.1000	A
Dose 2	5	1.000	A
Dose 1	5	0.9600	A

TC

Factor	N	Mean	Grouping
Control	5	2.040	A
Dose 3	5	1.820	A
Dose 2	5	1.7400	A
Dose 1	5	1.5200	A

UREA

Factor	N	Mean	Grouping
Dose 1	5	4.214	A
Control	5	4.058	A
Dose 3	5	3.326	A
Dose 2	5	3.1100	A

CRE

Factor	N	Mean	Grouping
Dose 1	5	127.54	A
Control	5	111.38	A
Dose 2	5	110.66	A
Dose 3	5	107.78	A

Pictorials