STUDIES ON ANTIHAEMORRHAGIC AND ANTIBACTERIAL PROPERTIES OF BULB JUICE, ETHANOL AND AQUEOUS EXTRACTS OF *ALLIUM SATIVUM* LINN. (LILLIACEAE) AGAINST MENINGEAL BACTERIA

BY

KOKORI, BAJEH TIJANI

DEPARTMENT OF PHARMACOLOGY & THERAPEUTICS

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY ZARIA,

NIGERIA.

AUGUST, 2014

STUDIES ON ANTIHAEMORRHAGIC AND ANTIBACTERIAL PROPERTIES OF BULB JUICE, ETHANOL AND AQUEOUS EXTRACTS OF *ALLIUM SATIVUM* LINN. (LILLIACEAE) AGAINST MENINGEAL BACTERIA

BY

Bajeh Tijani KOKORI, B. Sc BIOCHEM. (BUK) 1998

M.SC./PHARM.SCI./00774/2008-2009

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE DEGREE IN PHARMACOLOGY.

DEPARTMENT OF PHARMACOLOGY &THERAPEUTICS, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

AUGUST, 2014

DECLARATION

I hereby declare that this research work was carried out by me in the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences of Ahmadu Bello University Zaria, Nigeria under the supervision of Dr. N. M. Danjuma, Dr. J. I. Ejiofor (Department of Pharmacology and Therapeutics) and Dr. B. O. Olayinka (Department of Pharmaceutics and Pharmaceutical Microbiology). The work of other researchers were duly acknowledged and referenced accordingly. No part of this work has been submitted elsewhere for the award of a degree or diploma at any other University.

KOKORI, Bajeh Tijani _____ _____

Name of student **Signature** **Date**

CERTIFICATION

This Thesis entitled STUDIES ON ANTIHAEMORRHAGIC AND ANTIBACTERIAL PROPERTIES OF BULB JUICE, ETHANOL AND AQUEOUS EXTRACTS OF *ALLIUM SATIVUM* LINN. (LILLIACEAE) AGAINST MENINGEAL BACTERIA by Bajeh Tijani KOKORI, meets the regulations governing the award of Masters degree in Pharmacology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

…… .....……..…….
Dr. N. M. Danjuma Date
Chairman, Supervisory Committee

…… ……..
Dr. J. I. EJIOFOR Date
Member, Supervisory Committee

…… ……
Dr. B. O. Olayinka Date
Member, Supervisory Committee

…… ……
Dr. A. U. Zezi Date
Head of Department

…… ……..……..
Prof. A. A. Joshua Date
Dean, School of Postgraduate Studies

ACKNOWLEDGEMENT

My profound gratitude goes to my Creator, for His goodness, divine favour, spiritual guidance, strength, sustenance and for being my Supreme helper all through my academic pursuit.

My heartfelt gratitude goes to my supervisors Dr. N. M. Danjuma, Dr. J. I. Ejiofor and Dr. B. O. Olayinka for their encouragement and support during the course of this work. I appreciated the contributions of Dr. N. M. Danjuma and Dr. B. O. Olayinka in fine-tuning this work.

I also want to acknowledge Mal Kabiru Ibrahim (Pharmacognosy and Drug Development Dept.), Mal Bashir Musa (M.U.S.R.L.), Mal Bilal S. Abdullahi (Food Science & Processing Dept., IAR), Prof. Y.K.E. Ibrahim (Pharmaceutics and Pharmaceutical Microbiology Dept.), Dr. O. R. Momoh (Chemical Engineering Dept.), and Dr. Jamilu Ya'u (Pharmacology and Therapeutics Dept.) Pastor Ezekiel Dangana (Pharmaceutical Microbiology Dept.) all from ABU-Zaria; Mal Hussein Muhammed (N.A.R.I.C.T.) Bassawa, Zaria; Prof. M.K. Ajala (N.A.P.R.I. Shika), late Prof P.O. Okaiyeto (N.A.P.R.I. Shika) and Dr. Otaru S. M (N.A.P.R.I. Shika) for their encouragement and academic support;

I also appreciate the efforts and their support made for my academic tolerance and my inspiration boost. I really acknowledge Dr. A.A.Yusuf (Kongo Clinic, ABU), Dr. Garba Y. Itopa (ABUTH, Shika), Pharm. Salihu Abdul Nasiru, Pharm. John Nicholas

Obinna, Pharm. (Mrs) Yabo Phoebe Kato and Pharm. Kasim Muhammad for their moral support.

The Technologists in the Dept. of Pharmacology & Therapeutics (ABU, Zaria) for the technical assistance during the Laboratory work. May the Almighty Allah reward you all abundantly, Ameen.

Special thanks to Mr.Theophilus Mba and Mallam Suleiman AbdulYekini (ABUTH, Medical Microbiology Department), for their useful and valuable contributions during the Laboratory Research.

A million thanks goes to Sheikh Fauzul Azeem Cice, Mall. (Adayi) Salihu Gambari, Mall. Yakubu Raji Eganyin, My brothers - Ogbojo Isah Yakubu and Hassan O. Shaibu for their tireless financial and moral support.

My thanks also go to Alh. Bello Ovada (Sariki Yaki Palladan, Zaria), Mall. Muhammed Salau, Mall. Nasir Salawu, Hon. Momoh A. Asuva, Pastor Lasisi Onsachi, Mall. Muhammed Ohiani (NAMA, Zaria), Mall. Tanimu Garba, Pharm Ahmad Bin Yusuf, Late Mall. Momoh Abdulrahman, Mr. Timothy Kunle, and Mallam Abdullahi Ojapa for their numerous moral supports during my academic pursuit.

To these very special people in my life: my wonderful wife and our lovely children, thank you for your support, encouragement and sacrifices; thanks for standing by me, and with me under the sunshine and in rain, in storms and in the calm. You are

precious to me; you are Allah's gift to me. Thank you for making it easy for me to fulfill my clarion call. I love you.

I am not less grateful to those whose names are not listed here or who have helped me in one way or the other or whom I might have forgotten; King of Glory and the Beneficent will bless you all.

DEDICATION

This thesis is dedicated to Almighty Allah my Creator, my family and my loved ones.

ABSTRACT

Bacterial meningitis (BM) is a devastating air-borne infectious disease of humans that causes cellular hemorrhagic sepsis, metabolic, neurologic and endocrine disorders and constitutes a serious threat to public health. Where malnutrition is linked to BM, it is a great economic and societal burden accompanied with high morbidity and mortality rates in Sub-Saharan Africa including Nigeria. *A. sativum*, a medicinal food herb found in Northern Nigeria, has been used in folklore to treat infectious diseases locally including haemorrhagic sepsis and malnutrition. In the present investigation, the antihaemorrhagic and *in vitro* antibacterial activities of the juice (JEAS), ethanolic (EEAS) and aqueous (AEAS) bulb extracts of *A. sativum* were evaluated against bacterial meningitis pathogens. The methods employed in this study were validation of phytochemical screening which was done according to standard methods, determination of nutritional composition was carried out using analytical automated instruments (Atomic Absorption Spectrometers), investigation of antihemorrhagic activity was done using mean blood clotting time of wistar rats, and evaluation of *in vitro* antibacterial activities of the extracts against clinical isolates using agar-well diffusion and broth dilution methods. The clinical isolates of meningitis pathogens, *Streptococcus pneumoniae, Neisseria meningitides, Klebsiella pneumoniae, Haemophilus influenzae* and *Escherichia coli* were obtained from Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria. The phytochemical screening of *A. sativum* extracts (JEAS, EEAS and AEAS) revealed the presence of alkaloids, carbohydrates, cardiac glycosides, fats & oils, flavonoids, saponins and steroidal terpenoids. The results obtained as nutritional profiles from analytical automated machines analysis showed that *A. sativum* contained all classes of food nutrients such as carbohydrate, protein, fat and oils, dietary fibres, and

vitamins together with zeolite herbominerals (nanopharmacologic effects). The mean blood clotting time of the three extracts on afflicted fresh wounds on rat were also determined. JEAS and EEAS extracts were potent in both antihemorrhagic (0.94 ± 0.01 minutes), (0.99±0.04) and antibacterial activities while and AEAS (1.20±0.04) showed low activity, inhibiting the clinical bacterial isolates *Neisseria meningitides, Streptococcus pneumoniae, Haemophilus influenzae* and *Escherichia coli* with diameter of zone of inhibition ranging from 15-36 mm at concentrations of 10, 15, 20 and 25 mg/ml. It produced significant ($p<0.05$) antibacterial activity while EEAS and AEAS showed low activities, except *Klebsiella pneumoniae* which was resistant to the three extracts concentrations used. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs ranging between 0.04-1.56 mg/ml while MBCs was 0.10-2.50 mg/ml respectively. The findings from this study could be of interest and suggest the need for further investigations with a view to use the plant in novel drug development for BM therapy. The antihemorrhagic and antibacterial activities of the three extracts were statistically significant ($p < 0.05$). JEAS also exhibited shorter clotting time (Waller Duncan Test, SAS). The outcome of this study could therefore justify the ethnomedical and folkloric usage of *A. sativum* to treat bacterial meningitis locally.

TABLE OF CONTENTS

page

Title Page i

Declaration ii

Certification iii

Acknowledgement iv

Dedication vii

Abstract viii

Table of Contents x

List of Tables xiii

List of Figures xiv

List of Plates xv

List of Appendices..... xvi

Acronyms and Abbreviations..... xvii

1.0 **INTRODUCTION**..... 1

1.1 **Background for the Study**..... 1

1.2 **Statement of the Research Problems** 3

1.3 **Justification for the Study**..... 4

1.4 **Aim and Objectives of the Study**..... 6

1.5 **Statement of Research Hypothesis**..... 6

2.0 **LITERATURE REVIEW** 7

2.1 **The Epidemiology of Bacterial Meningitis**..... 7

2.2 **The Pathophysiology of Bacterial Meningitis**..... 9

2.3 **The Common Signs and Symptoms of Bacterial Meningitis**..... 11

2.4 **Aetiologic Factors of Bacterial Meningitis**..... 12

2.5 **Treatment of Haemorrhage and Bacterial Meningitis**..... 13

2.5.1 Steps in Haemostasis……13

2.5.2 Antihaemorrhagic Agents……14

2.6 **Antibacterial Chemotherapeutic Agents**……...16

2.6.1 Mechanisms of Action of Antibiotic Drugs……..18

2.6.2 Antibiotic Used in the Study……29

2.7 **Description and origin of *A. sativum* Plant** …….. 33

2.8 **Folkloric and Ethnomedical Uses of *A. sativum***…….…… 34

2.9 **Some Scientific Validations of Use of *A. sativum***…….…… 35

3.0 **MATERIALS AND METHODS**…… 36

3.1 **Equipments and Instruments**…… 36

3.2 **Drugs, Solvents and other Materials** …… 36

3.3 **Collection and Identification of Plant Material**……37

3.4 **Extractions of Plant Material** …….. 37

3.5 **Solutions** ……. 38

3.6 **Test Clinical Bacteria Isolates**.....38

3.7 **Laboratory Animals** ……..... 38

3.8 **Phytochemical Screening of *A. sativum* Extracts**…….. 39

3.9 **Nutritional Composition and Proximate Values of extract JEAS** ……. 40

3.10 **Determination of Blood Clotting Time Effects (Antihaemorrhagic) of *A. sativum* Extracts in rat Wounds** ……..…..... 41

3.11 **Determination of Antibacterial Activity of *A. sativum* Extracts**…..…….42

3.12 **Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**……..43

3.13 **Statistical Analysis** ……..44

4.0 **RESULTS** …… 45

4.1 **Extraction of Plant Material and Percentage Yield** …… 45

4.2 **Phytochemical Constituents of *A. sativum* Extract**…… 46

4.3 **Analysis of Nutritional Compositions of *A. sativum* Extract** …… 47

4.4 **The Blood Clotting Effects (Antihaemorrhagic) of *A. sativum* Extract in Rat Wounds**……..……48

4.5 **Antibacterial Activity of Extracts of *A. sativum***…….…….. 49

4.6 **Minimum inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. sativum* extracts**……..…….. 50

5.0 **DISCUSSION.** …… 55

6.0 **SUMMARY, CONCLUSION AND RECOMMENDATIONS**……..... 64

6.1 **Summary**…… 64

6.2 **Conclusion**……..... 64

6.3 **Recommendations** …….. 65

REFERENCES…… 66

APPENDICES.....75

LIST OF TABLES

Table **Page**

Table 4.1 **Percentage yield of *A. sativum* extracts**…… 45

Table 4.2 **Phytochemical Constituents of *A. sativum* extracts**…… 46

Table 4.3 **Nutritional Compositions and Proximate Values of *A. sativum* Extract**…… 47

Table 4.4 **Blood Clotting as Anti-haemorrhagic Effects of *A. sativum* Extract on Rat wounds**…….. 48

Table 4.5 **Inhibitory Effects of *A. sativum* Extracts**……49

Table 4.6 **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Standard Drug (5mg/ml)**……....50

Table 4.6.1 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. sativum* Extracts (10mg/ml)…..……..51

Table 4.6.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. sativum* Extracts (15mg/ml)……..52

Table 4.6.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. sativum* Extracts (20mg/ml)……..……...53

Table 4.6.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. sativum* Extracts (25mg/ml)……..54

LIST OF FIGURES

Figures **Page**

Fig 2.1. **Target of antibacterial medications in pharmacotherapeutics**…….19

Fig 2.2 **Antibacterial medications that interfere with cell wall biosynthesis**…....20

Fig 2.3. **The β-lactam ring of penecillins and cephalosporins**…….....21

Fig 2.4. **Lysed bacterial cell**…..…….22

Fig 2.5. **Inhibition of folate pathway**……..……...24

Fig 2.6. **Antibacterial drugs that inhibit protein synthesis**..……..……..25

Fig 2.7. **Antibacterial drug inhibiting cell membrane synthesis**..…….26

Fig 2.8. **Chemical structure of cefuroxime axetil**……..……..30

LIST OF PLATES

Plates **Page**

Plate 1. **The Garlic Plant in Its Natural Habitat**…..……. 34

Plate 2 ***A. sativum* extracts antibacterial activity using agar well (*Hi*)**…… 77

Plate 3 ***A. sativum* extracts antibacterial activity (*Sp, Ec* and *Kp*)**…….. 78

Plate 3 ***A. sativum* extracts antibacterial activity (*Nm*)** …..…….7

LIST OF APPENDIX

Appendices **Page**

Appendix1. **Effects of nutrition powdered innate immunity (first line of defense) through biochemical complements**……..75

Appendix2. **Second line of immune defense system (Adaptive immunity) provocation due to breaching of innate immunity as a result of malnutrition**…….76

ACRONYMS AND ABBREVIATIONS

AEAS - Aqueous extract of *Allium sativum*

ABUTH - Ahmadu Bello University Teaching Hospital

ABUZ - Ahmadu Bello University, Zaria

AS - *Allium sativum*

ANOVA - Analysis of Variance

BM - Bacterial meningitis

BN - Batch number

CFU - Colony forming unit

CSF - Cerebrospinal fluid

DH_2O - Distilled water

Defg - Def gene for bacterial growth and survival

DF - Degree of freedom

DIC - Disseminated intravascular coagulation

DNA - Deoxyribonucleic acid

dTMP - Deoxythymidine monophosphate

dUMP - Deoxyuridine monophosphate

EEAS - Ethanolic extract of *Allium sativum*

IADHS - Inappropriate antiduretic hormone secretion

IL - Interleukin

IUPAC - International union of pure and applied chemistry

JEAS - Juice extract of *Allium sativum*

KR - Killing rate

Linn - Linnaeus

MAP - Meningitis associated paralysis

MBC - Minimum bactericidal concentration

MHA - Mueller-Hinton agar

MDGPs- Millennium development goal projects

MIC - Minimum inhibitory concentration

mRNA - Messenger ribonucleic acid

MRSA - Methicillin resistant-staphylococcus aureus

NA - Nutrient agar

NA - No activity

NB - Non bactericidal

NB - Nutrient broth

OMP - Outer membrane proteins

PABA - Para-aminobenzoic acid

PBPS - Penecilin-binding-proteins

PVP - Polyvinylpyrorridone

RNA - Ribonucleic acid

SEM - Standard error of the mean

TB - Tuberculosis

tRNA - Transfer ribonucleic acid

TNF - Tumour necrosis factor

TR - Timing rate

UDN-N-AMPP -Uridine dinudeotide-*N*-acetylmuramylpentapeptide

UN - United Nations

USA - United States of America

USDA- United States drug and administration

UK - United Kingdom

USP - United States Pharmacopoeia

VAP - Vaccine-associated paralysis

VREF - Vancomycin-resistant enterococcus faecium

VRSA - Vancomycin-restant staphylococcus aureus

WHO – World Health Organization

WHO – BS - World Health Organization-Biological Standardization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background for the Study

Bacterial meningitis (BM) is a devastating air-borne infectious disease of humans, and it is of huge health burden as it can easily kill or maim when meningeal pathogens invaded blood stream. The BM causes cellular hemorrhagic sepsis, metabolic, endocrine and neurologic disorders. This burden of the disease is highest in the developing countries and especially in the immunity compromised rural populations. Malnutrition linked to BM is a great economic and societal burden in developing countries of Africa including Nigeria. As with most pathological disorders of the Sub-Saharan African countries often due to malnutrition that stems from poverty, consumption of foods deficient of vital nutrients unavailability and/or high cost of the right sorts of food especially in rural communities (World Health Organization, WHO, 2007; United Nations, UN, 2012). Nutritional deficiencies or lack of vital nutrients in diets often leads to the development of certain physiologic and biochemical malfunctions that weakens the immune defense and predisposes the vital organs of the body to damaging infections. Meningococcal meningitis and its epidemics (cerebrospinal fever) have been reported to have about 95% links to malnutrition (UN, 2012). BM is a serious bacterial illness characterized by the inflammation of any or all of the membranes (*dura mater*, arachnoid and *pia mater*) enclosing the central nervous system (brain and spinal cord) and which often leads to the damage of the blood vessels and meninges (Saez-Llorens and McCracken, 2003; Van de Beek *et al*., 2006). It is mostly endemic during the hot dry season typical of the tropical Sub-Saharan African countries, the hot climate of which favours the

breeding of the meningococcal organisms that infect the meninges (Dossett, 2001; Okoko *et al*., 2009). Inflammation of the meninges causes leakage of the infected cerebrospinal fluid (CSF) and alteration of the brain system (cognitive deficit) giving rises to meningism (stiff neck, severe headache, fever, rashes, shortness of breath or noisy breathing, hydrocephalus (swelling or oedema of the brain microglia and astrocytes due to inappropriate antidiuretic hormone secretion etc).

Meningococcal-induced haemorrhage is a complication of malnutrition and a serious health burden of economic and societal especially in the impoverished rural communities of Sub-Saharan Africa and has actually been noted to be of high impact in the Northern States of Nigeria probably with regards to the exceptionally hot dry weather and climate of this part of the country (Peltola, 2000; Schromm *et al*., 2000; WHO, 2009).

Traditional medicine constitutes an important source of drugs for ethnopharmacological investigation. Various medicinal food-plants and animal products-supplements are available for use in certain immune-deficiency disease conditions related to malnutrition such as hemorrhagic sepsis. Furthermore, phytotherapy still remains a habitual part of health care system wholly or in part especially in rural communities (Derrida, 2003; Sofowora, 2008). *A. sativum* (AS) is one of such medicinal food-plant that has a long history of use as food condiment and it also has a high value of use traditionally for many ailments including typhoid fever, ulcer, cholera, dysentery etc and thus, there is a need to investigate its specific *in vitro* antibacterial activity on meningeal bacterial organisms as well as its *in vitro*

antihaemorrhagic activity in condition of meningococcal-induced cellular injuries having been known to boost the immune system (Rukangira, 2001; Rojas *et al*., 2006; Ncube *et al*., 2008).

1.2 Statement of Research Problems

Bacterial meningitis is a complication of malnutrition and a serious public health burden of high morbidity and mortality especially in the impoverished rural communities of Sub-Saharan Africa and has actually been noted to be of high impact in the Northern States of Nigeria probably with regards to the exceptionally hot climate change of this part in the country that favours the breeding of the infecting organisms (Okoko *et al*., 2009; Onipede *et al*., 2009; O'Brien *et al*., 2009).

Thousands of people worldwide also die of cerebrospinal meningitis fever as a result of disseminated intravascular coagulation of the blood cells often caused by certain meningococcal organisms and the associated inability of the blood cells (haemoglobin) to transport oxygen (methaemoglobinaemia) (Van de Beek *et al*., 2006; Chan *et al*., 2007; Pilkhwal *et al*., 2010).

Aside of the massive side effects associated with the conventional antibiotics, there has also been the emergence of resistant strains of the infecting organisms probably from the frequent irrational use of the available antibiotics to treat conditions of unknown aetiology, and often, resistance hampers the control of bacteria in general and tends to increase cost of healthcare services (Nikaido and Pages, 2012).

Antibacterial agents and vaccines meant to reduce morbidity and mortality of BM as modern therapy are availabe. Despite all these efforts put in place, BM remains associated with unacceptable rate of high morbidity and mortality in Sub-Saharan Africa, including Nigerian Northern States (Michael *et al*., 2002).

1.3 Justification for the study

Most rural dwellers are less privileged in so many things such as having good modern healthcare facilities and services as well as good transportation systems in emergencies, and affordability of effective medicines for their ailing conditions (WHO, 2010; Rukangira, 2001).

The main ideas of the uses of medicinal plants are for prevention and maintenance of normal healthcare rather than resort to treatment in disease state. However, the discovery that a plant-herb commonly consumed in food has nutritional medicinal potential as well as antibacterial and antihaemorrhagic properties will for sure be of high value in the line of reducing the frequent bacterial meningitis emergencies of the rural communities (Fahey, 2005; Derrida, 2003).

Crises of malnutrition in rural communities with its associated weakening of the immune system that leads to invasion of bacterial infections is a cause for concern that warrants a search for food plant-herb that can boost the immune system and help reduce spontaneous bursting of blood vessels, bleeding of blood vessels and bacteria-induced cellular injuries (Fahey, 2005; UN, 2012).

High cost of acquiring synthetic antibiotics and the inconveniences of seeking for effective treatment due to using exorbitantly expensive drugs make life difficult for people, considering the economic status of an average Nigerian as a low income earner (Derrida, 2003; Rukangira, 2001).

The loss of effectiveness of chemotherapy due to bacterial resistance, constitute the greatest threat to the control of bacterial meningitis (BM). Moreover re-emergence of BM persists in epidemic meningitis belt of Africa. Therefore, to overcome BM, new knowledge of natural products against BM pathogens is urgently needed; especially new drugs are required for BM mitigation which differ from existing medicines (Rojas *et al*., 2006; Nikaido and Pages, 2010).

A. sativum (AS) has a long history of use as food condiment and also a high value of use traditionally for many ailments including antimicrobial effects. However, its specific activity on meningeal bacteria as well as antihaemorrhagic activity in condition of meningococcal-induced cellular injuries has actually not been reported in literature. Also, a scientific validation of this utilization has not been previously made to the best of my knowledge.

1.4 Aim and Objectives of the Study

The main aim of this study is to evaluate the antihaemorrhagic and antibacterial activities of the fresh juice (JEAS), ethanolic (EEAS) and aqueous (AEAS) extracts of *A. sativum* on bacterial meningitis pathogens.

Specific objectives:

1. To validate the phytochemical screening of the extracts of AS (JEAS, EEAS and AEAS).
2. To determine the nutritional composition and proximate values of JEAS.
3. To investigate the blood clotting time (antihaemorrhagic effect) of the extracts (JEAS, EEAS and AEAS) on wounds inflicted on wistar rats.
4. To evaluate the *in vitro* antibacterial activity of the extracts (JEAS, EEAS and AEAS) on clinical isolates of bacterial meningitis pathogens (*Neisseria meningitides*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae*).

1.5 Hypothesis

The *A. sativum* bulb extracts (JEAS, EEAS and AEAS) contain valuable pharmacologically active principles for antihaemorrhagic and antibacterial properties against bacterial meningitis pathogens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Epidemiology of Bacterial Meningitis

For decades, exclusive worldwide bacterial meningitis (BM) epidemic is estimated to be about 1.2 million cases with 135,000 deaths. BM is now 'top 10' infectious disease that causes death worldwide and half of the survivors deformed (Michael *et al*., 2002). Explosive annual epidemic of meningeal bacteria occurs during the hot dry season in Sub-Saharan African countries and during the cold rainy season in western countries (WHO, 2009; Okoko *et al*., 2009; IRIN African News, 2009). At present in Africa, the incidence cases range from 100 to 1000 per 100,000 populations of people against 20 per 100,000 populations in the western world. At other times, the incidence rate-range is only 10 to 20 per 100,000 for Sub-Saharan African countries and 1 to 5 per 100,000 for western countries (USA, UK and Europe) (Stephens *et al*., 2007; WHO, 2009). Although a yearly average incidence of 333,000 cases was reported for Sub-Saharan African countries generally including Benin, Burkina Faso, Cameroon, Central Africa, Chad, Congo, Ethiopia, Ghana, Nigeria, Niger, Mali, Togo and Tunisia. Nigeria alone had an incidence case of about 24,000 subjects in the 2009 epidemic season when the incidence rate in USA was only about 2,600 (Onipede *et al.,* 2009; WHO, 2009). In general, the fatality cases range from 10-20% (about 33,300 -66,600) of the affected population in both Sub-Saharan African countries and the western countries (about 260 -520). However, an estimated death of about 1,500 people was reported in Nigeria in the 2009 epidemic season with many of the survivors deformed (WHO, 2009). Nigeria was in-fact cited as one of the meningitis belt zone of the Sub-Saharan African countries during the 64th world health assembly meeting in Geneva by Bill and Melinda Gate foundation. The foundation had tasked

African leaders to save 4 million lives by year 2015 and 10 million lives by year 2020; as part of millennium development goal projects (MDGPs) against BM in African region (Bill and Melinda, 2011). Children and young adults are usually highly affected and approximately 80% cases of meningitis occur in children under the age of 5 (Saez-Llorens and McCracken, 2003; Okoko *et al*., 2009).

As for the prevalence of meningeal bacterial organisms, the potential of epidemic virulence of meningeal bacteria organism in Nigeria varies with the geographical location, time of season, climate and pathogenic serotype strains of the causative organism (Lovera and Arbo, 2005). Six subspecies serotypes of *meningococci* (A, B, C, W-135, X and Y) have been clinically recognized in Sub-Saharan African countries of which serotypes A and C *meningococci* were found to be more prevalent and predominant among other serotypes which often occur as co-morbid infections (WHO, 2009). The epidemics of Sudan in 2006 was of serotypes W-135 and X (Segal and Pollard, 2004; Tunkel *et al*., 2004; WHO Bulletin, 2009). The most prevalent meningeal bacteria organism in Nigeria is of the serotype A and C strains of *Neisseria meningitides* seen abundantly during the harmattan period when humidity is very low, usually around November-June and which is the breeding time of the organisms. *Neisseria meningitides* is the most implicated bacterial organism that causes meningitis and constitutes about 80% of the epidemic cases in Africa (Molesworth *et al*., 2003; Sultan *et al*., 2005; Onipede *et al*., 2009). Other bacterial organisms (*Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*) also cause meningitis to a lesser extent (about 20% of the epidemic cases) (Onipede *et al*., 2009; Okoko *et al*., 2009). Thus, these five bacterial organisms are those mostly known to be involved in meningitis, systemic

septic-inflammations and hemorrhage in Sub-Saharan Africa including Nigeria (Onipede *et al*., 2009). Bacterial meningitis caused by *H. influenzae* and *meningococci* have a better prognosis than of *S. pneumoniae* and *E.coli* that mainly attack neonates and growing children. The group B *streptococci* (subtype III) occur mainly during the first week of life (newborns), while *Escherichia coli* and *Klebsiella pneumoniae* are of both newborns and growing children less than five years of age. *Haemophilus influenzae* type B, *Neisseria meningitides* (meningococci) and *Streptococcus pneumoniae* affect mostly the adults, but people over 50 yrs have increased risk of *Escherichia coli* (Saez-Llorens and McCracken, 2003; Van de Beek *et al*., 2006; Cuevas *et al*., 2007).

2.2 The Pathophysiology of Bacterial Meningitis

Usually the weakening or slacks in the functions of the immune defense system predisposes the body system to damaging opportunistic infections. Bacterial infection in particular often leads to septicaemia or persistent multiplication that traumatizes the blood vessels. The bacterial endotoxins tend to rupture its traumatized blood vessels and often cause bleeding and shunt in the flow of blood to the vital organs thus disrupting their functions (vital organs shot down) (Donnet, 2007; Jeeri and Thiombiano, 2008; Tebruegge and Curtis, 2008). The endotoxin of both the gram negative contains coat with arabinoside peptidoglycan and lipopolysaccharide and gram positive coat with lipoteichoic acid and muramyldipeptide. *Neisseria meningitides* often get tangled with blood vessels resulting in their spontaneous bursting, or rupture of the blood vessel which can cause gushing out of blood (haemorrhage) accompanied with the weakness of collagen capacity (Tebruegge and Curtis, 2008). There are some of the bacterial organisms that also have the tendency of producing clotting enzymes (coagulases) which down regulates the clotting

mechanisms by forming coagulums (conversion of thrombin to fibrin) resulting in disseminated intravascular coagulation (DIC) of blood cells into thick solid mass (scabs) that impede blood flow and shuts down the vital organs (Jeeri and Thiombiano, 2008; Walker and Whittlesea, 2008; Corey *et al*., 2011). The gram negatives of the bacterial organisms coagulate the blood faster and these coagulums may lead to stroke and failure of vital organs. Sometimes, the bleeding blood cells tend to form pus (haemorrhagic sepsis) and in union with circulatory hindrances and excessive tension on blood pressure. They produce generalized inflammations particularly meningoencephalitis, arthritis, cellulitis, vasculitis, osteomyelitis etc), and other signs and symptoms of meningitis (meningism) such as stiffness especially of the neck. It gives rise to neuroinflammation in which the brain responds to infections, diseases and cellular injuries as post infectious attack to form meningitis associated paralysis (MAP) (Tebruegge and Curtis, 2008). In the extreme conditions involving intravascular coagulations, organ failure and / or sudden death may occur related to the rapid shutdown of the vital organs. Haemorrhage is a severe form of blood loss that can cause general shock as a result of reduced tissue perfusion and / or ischaemia (Corey *et al*., 2011; Hackam *et al*., 2011). Meningeal bacteria causing reduction in the levels of blood clotting cells (platelets), this leads to severe erythematous purpura, DIC and uncontrollable bleeding which can be internal haemorrhage in the vital organs of the body (Tebruegge and Curtis, 2008). These blood clots can reduce or block blood flow through the body blood vessels due to bacteria coagulases lead to the damage and sudden death of body's organs. In DIC, the increased clotting uses up platelets and clotting factors in the blood, it deregulates normal physiologic mechanisms of the blood. Furthermore, it can cause internal and external bleeding in meningitis patients mainly infants (Hackam *et al*., 2011). Thus, internal bleeding occurs inside the body while external bleeding occurs underneath or from the skin or

mucosa. These meningitis pathogens cause sepsis of the blood, fever, diarrhoea and sudden death (Jeeri and Thiombiano, 2008; Hackam *et al*., 2011).

The inflammatory episodes (body defense mechanism) is usually as a result of the release of inflammatory mediators like macrophages, cytokines, interleukins (IL 1 & 6), tumour necrositic factors (TNF), enhanced nitric oxide production, thrombopoietin from liver etc which flood-poisons the nerve system causing ischaemia. This provoke

defense reactions as the immunologic response of the body against bacterial endotoxins and it often leads to neural irritation especially of the sub-arachnoid space, diffused inflammation of neural and vascular structures including cerebral vasculitis, cerebral venous thrombosis (blood clots in veins) as well as the abnormalities of the cranial nerves. When the cranial nerve supply is hampered or disrupted, the activities or functions of its supply zones - head, neck, facial muscles, visual and hearing functions also become impaired and most times, the visual symptoms and hearing loss persist even after the episode of meningitis (Ginsberg, 2004; Tebruegge and Curtis, 2008; O'Brien *et al*., 2009).

2.3 The Common Signs and Symptoms of Bacterial Meningitis

Inflammation of the meninges causes leakage of the infected CSF-fluid and alteration of the brain system (cognite deficit) giving rise to meningism (stiff neck, severe headache, fever, rashes, shortness of breath (from depletion of the lung's surfactants, phospholipid, lecithin, and phosphotidylcholine) or noisy breathing (from intercoastal muscle collapse with myoglobin oxygen deficit), hydrocephalus (swelling or oedema of the brain astroglial cells react to bacterial endotoxin that cause inappropriate antidiuretic hormone secretion, IADHS) and neuronal apoptosis; and from deficient blood glucose and vital minerals, shock with coma, vascular collapse, fainting also

occur which could worsen the pathological conditions (Tebruegge and Curtis, 2008; Onipede *et al.,* 2009).

Other signs and symptoms include:

Pains (abdominal, chest), swelling, hemoptysis (bloody vomitus), rashes, photophobia (light intolerance),hyperthermia, hypoglycemia, convulsion/seizures, phonophobia (high noise intolerance), drowsiness, confusion, clouded vision, loss of consciousness, arthalgia, altered mentation, sight and hearing loss and crippled limbs (Tebruegge and Curtis, 2008; Onipede *et al.,* 2009).

In children, there may be high pitched cry, loss of appetite, bulging of the fontanelle (forehead), cold extremities (hypothermia), diarrhoea, cyanosis, methaemoglobinemia (IRIN African News, 2009; Onipede *et al*., 2009).

2.4 Aetiologic Factors of Bacterial Meningitis

- Malnutrition
- Climate change
- Overcrowding (as in crowded quarters, or gatherings with infected persons who most times are asymptomatic carriers of the pathogens)
- Deficient immune system
- Poor sanitation (environmental hygiene) that breeds the causative organisms
- Symptoms of inappropriate antidiuretic hormone secretion (SIADHS) in bacterial endotoxin-induced oedema of the body due to endocrine crises (Molesworth *et al*., 2003; Thomson *et al*., 2006; UN, 2012).

2.5 Treatment of Haemorrhage and Bacterial Meningitis

Haemostasis is the process of blood clot formation to stop bleeding at the site of vascular injury. The haemostatic system maintains the integrity of the vascular complex network of cellular, ligand receptors and interacts enzymatically. The agent used to keep the blood within a damaged blood vessel is called antihaemorrhagic to prevent bleeding and save from shock i.e. it antagonizes haemorrhage (bleeding). It is essential that blood remain fluid within the circulation but clots at sites of vascular injury to prevent blood loss and general shock (Joseph, 2000).

2.5.1 Steps in Haemostasis

- It is the first stage of wound healing. This involves blood changing from a liquid to a gel. Intact blood vessels are central to moderating blood's tendency to clot. The endothelial cells of intact vessels prevent blood clotting with a heparin-like molecule thrombomodulin, and prevent platelet aggregation with nitric oxide and prostacyclin.
- When endothelial injury occurs, the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von Willebrand and factor which initiate the maintenance of haemostasis after injury.
- Haemostasis has three major steps:
    1. Vasoconstriction (vascular spasm).
    2. Temporary blockage of a break by a platelet plug, and
    3. Blood coagulation or formation of a fibrin clot.

2.5.2 Antihaemorrhagic agents

Amicar

Instat

Ethicon 360

Evicel fibrin sealant

Vitamin K

Providone iodine

2.5.2.1 Amicar (Aminocaproic acid)

It is a derivative of amino acid lysine can either be injected, oral solution and tablets. It also acts as antifibrinolytic agent by inhibitory plasminogen activators which have fibrinolytic properties. The inhibitory effects of amino caproic acid appear to be exerted at the site of vascular injury to set vascular spasm.

Mechanism of Action: It binds reversibly to the kringle dormain of plasminogen and block binding of plasminogen to form fibrin and its activation to plasmin thereby promotes haemostasis (stops bleeding) or it inhibits activation of plasminogen activators which reduces blood loss (Benneth and Brown, 2008).

2.5.2.2 Instat (Avitene, Microfibrillar bovine derived)

It is a collagen absorbable haemostat that promotes haemostasis and wound healing, prevents bleeding and fastens clotting mechanism.

Mechanism of Action: The contact of instat with a bleeding surface collagen haemostat attracts platelets. Then adhere to its fibrils and undergo the release of its content. This triggers aggregation of the platelets into thrombin to form fibrous mass;

initiating the formation of a biochemical and physiologic plate plug (Bennett and Brown, 2008).

2.5.2.3 Ethicon 360 (Haemostatic matrix surgicel)

It is an absortable haemostat. It is a plant-base product used to control bleeding either surgical operation or accidental trauma with wide fresh wound. It interacts with the phospholipid around the injured area. It has astringent property and promotes haemostasis to form clot and seal the vascular tissue injured gap.

Mechanism of Action: It bind the injured vascular tissue, initiates and accelerates haemostasis, seal the injured gap with clot, stops bleeding and promote wound healing (Bennett and Brown, 2008).

2.5.2.4 Evicel fibrin sealant (Human glue)

It consist fibrinogen and thrombin contained in two syringes, the tips of which for a common port. It allows delivery of the two components to a bleeding point where fibrinogen converts to fibrin at a rate determined by the concentration of the thrombin. The fibrin glue can be used to secure surgical haemostasis, on a large raw and fresh wide wound surface. It also prevents external oozing of blood in patient.

Mechanism of Action: It is an adjunct to haemostasis for use in patient to prevent bleeding and control wound healing (Bennett and Brown, 2008).

2.5.5.5 Vitamin K (Koagulation vitamin, Phytomenadione)

It is essential for normal coagulation. Vitamin K_1 (phylloquinone) is widely distributed in plants and K_2 includes vitamin synthesized in the alimentary tract by bacteria to form menaquinones. Therefore, vitamin K is necessary for the final stage

in the syntheses of six coagulation-related proteins in the liver. The coagulant factors are II (Prothrombin), VII, IX and X with two others committed steps.

Mechanism of Action: vitamin K is an essential cofactor for the gamma – carboxylase enzymes which promotes the hepatic formation of active prothrombin (factor II). *Therapeutic Uses*: For the treatment of haemorrhage condition in infants, antidote for coumarin anticoagulant). Phytomenadione (Konakion) is preferred for its more rapid action, dose regimen vary according to the degree of urgency (Chen *et al.*, 2002).

2.5.2.6 Pyodine, Butadine, Pharmadine (Providone-iodine)

It is a stable chemical complex of polyvinylpyrrolidone (povidone, PVP) and elemental iodine. It contains from 5.0% to 12.0% available iodine. Povidone-Iodine is a broad spectrum antiseptic and astringent for topical application in the treatment and prevention of infection in wounds. Maybe used as first aid for minor cuts, grazes, burns, abrasions and blisters. It is an effective broad-spectrum bactericide and effective against yeasts, moulds, fungi, viruses and protozoans.

Mechanism of action: Povidone-iodine is a broad spectrum microbicide that destroys microbial protein and DNA by penetrate the cell wall of organism and exert astringent. It also inhibits neuraminidases and receptor binding inhibition (Rabih *et al.*, 2010).

2.6 Antibacterial Chemotherapeutic Agents

Chemotherapy refers to the control of any disease using drugs. However, the term chemotherapy is usually associated with the treatment of cancer by anti-tumor drugs. In the case of infectious disease, this involves the use of drugs that will kill or limit the growth of microorganisms. Until the early part of the century, no effective antibacterial chemotherapeutic drugs existed. For such a drug to be effective, it must

be able to kill or limit the growth of microorganisms without severely affecting cells of the host. This is referred to as selective toxicity. For bacteria, differences in structural components (such as the cell wall, minor differences in the ribosomes and RNA polymerase) are usually what is "attacked" by the chemotherapeutic drug (Overington *et al*., 2006).

The term "antibiotic" was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This original definition excluded naturally occurring substances that kill bacteria but are not produced by microorganisms (such as gastric juice and hydrogen peroxide) and also excluded synthetic antibacterial compounds such as the sulfonamides. In common usage, an antibiotic is a substance or compound that kills bacteria or inhibits their growth (Davey, 2000). Antibiotics belong to the broader group of antibacterial compounds, used to treat infections caused by microorganisms. With advances in medicinal chemistry, most antibiotics are now semisynthetic,modified chemically from original compounds found in nature (von Nussbaum *et al*., 2006), as is the case with beta-lactams (which include the penicillins, produced by fungi in the genus *Penicillium*, the cephalosporins, and the carbapenems). Some antibiotics are still produced and isolated from living organisms, such as the aminoglycosides, and others have bseen created through purely synthetic means: the sulfonamides, the quinolones, and the oxazolidinones. In addition to this origin-based classification into natural, semisynthetic, and synthetic, antibiotics may be divided into two broad groups according to their effect on microorganisms (Calderon and Sabundayo, 2007). Those that kill bacteria are bactericidal agent, whereas those that only impair bacterial growth are known as bacteriostatic agents.

Many treatments for infections prior to the beginning of the twentieth century were based on medicinal folklore. Treatments for infection in ancient Chinese medicine using plants with antimicrobial properties were described over 2,500 years ago (Lindbad, 2008). Many other ancient cultures, including ancient Egyptians and ancient Greeks used molds, honey and plants to treat infections (Madigan and Martinko, 2005). The discovery of the natural antibiotics produced by microorganisms stemmed from earlier work on the observation of antibiosis between microorganisms. Louis Pasteur observed that, "if we could intervene in the antagonism observed between some bacteria, it would offer 'perhaps the greatest hopes for therapeutics (Kingston, 2008). Synthetic antibiotic chemotherapy as a science and the story of antibiotic development began in Germany with Paul Ehrlich, a German medical scientist in the late 1880 (Calderon and Sabundayo, 2007). Scientific endeavours to understand the science behind what caused these diseases, the development of synthetic antibiotic chemotherapy, and the isolation of the natural antibiotics marked milestones in antibiotic development (Overington *et al.*, 2006).

2.6.1. Mechanisms of Action of Antibacterial Drugs

The mechanisms of action include the following:

Inhibition of cell wall synthesis

Inhibition of protein synthesis

Inhibition of nucleic acid synthesis

Inhibition of metabolic pathways

Interference with cell membrane integrity

Inhibition of Lipoteichoic acid

Inhibition of fatty acid biosynthsis

Inhibition of thymidylate synthase

Inhibition of DNA helicases

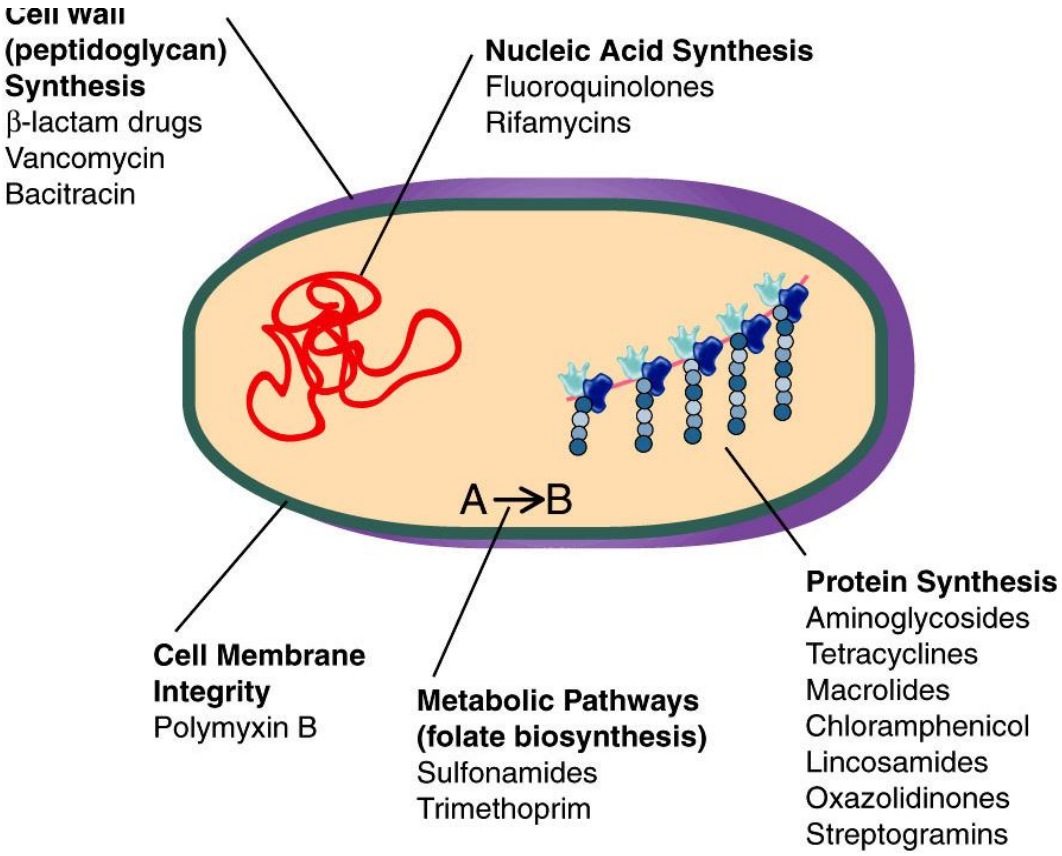


Fig 2.1: Target of antibacterial medications in pharmacotherapeutics.

Source: Hugo and Russell (2007).

Bacteria cell wall is unique in structure due to the presence of peptidoglycan. A number of medically useful drug interfere with cell wall biosynthesis, among these are penicillin, monobactam, carbepene, bacitracin and vancomycin. All these drug

shared chemical structure called β - lactam ring.This irreversibly inhibit enzyme involved in the final step of cell wall synthesis.

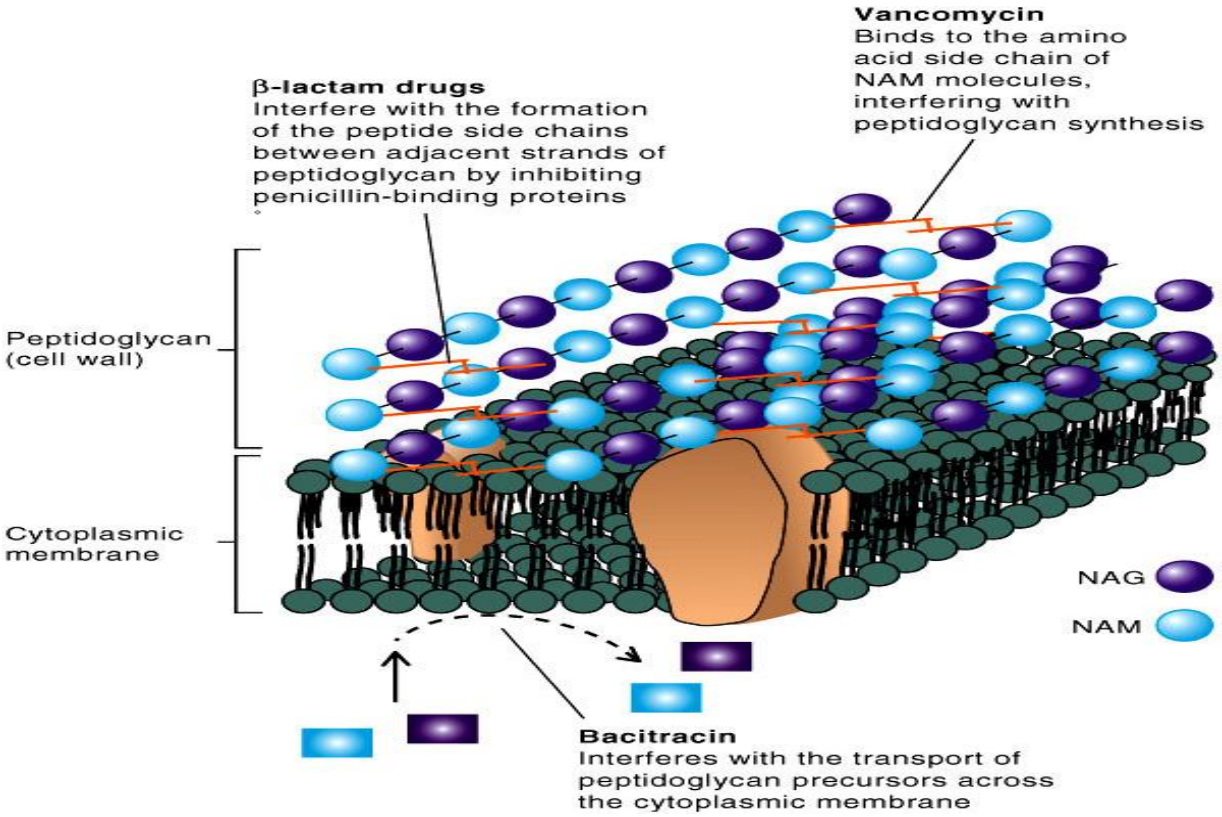


Fig 2.2: Antibacterial medications that interfere with cell wall biosynthesis.

Source:Hugo and Russel (2007)

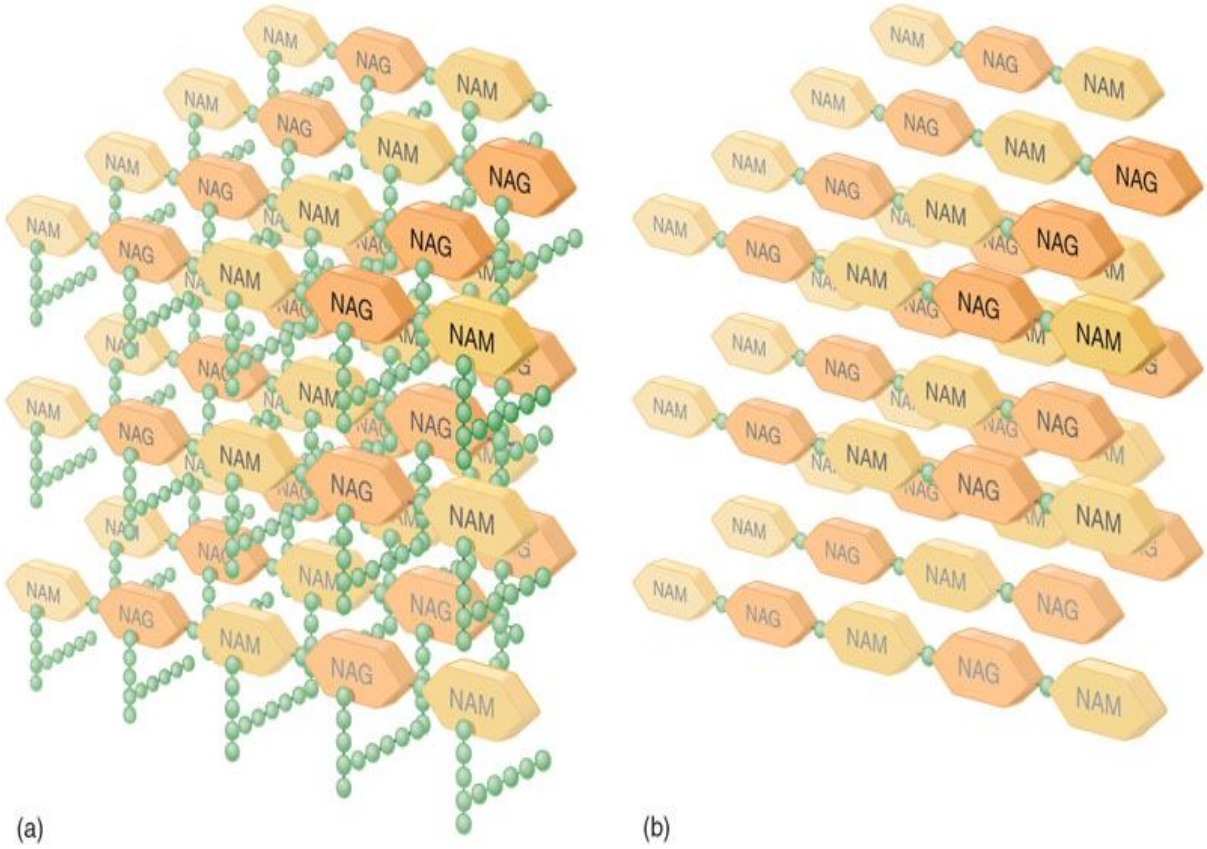


Fig 2.3: The β - lactam ring of penicillins and cephalosporins

Source: Prescott *et al.,* (2007).

Part of the drugs group called β–lactams have shared chemical structure called β-lactam ring.This causes the cell wall to develop weak points at the growth sites and become fragile. The weakness in the cell wall causes the cell to lyze.

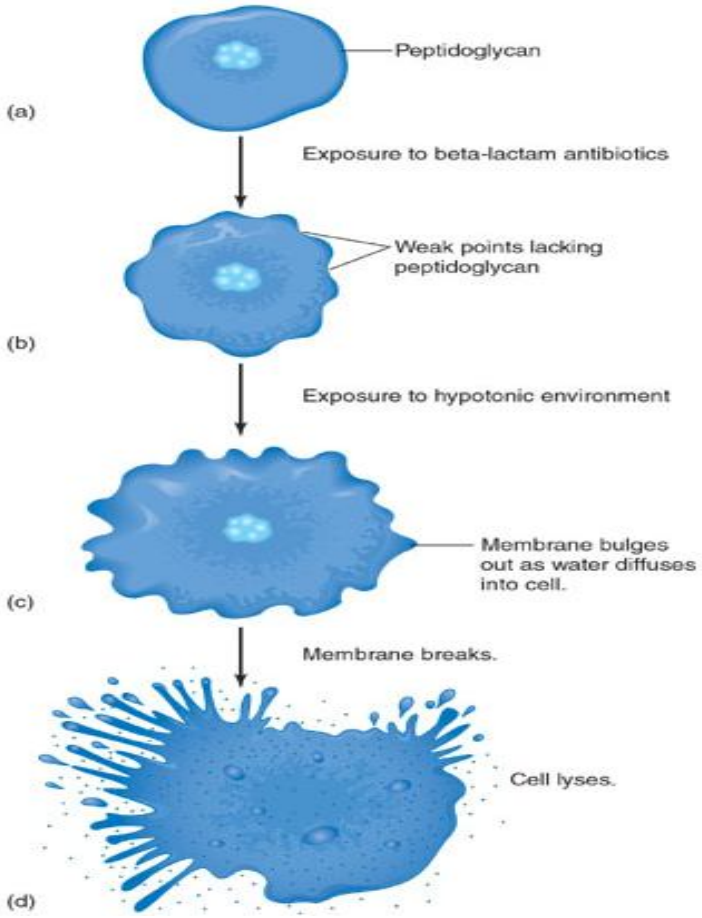


Fig 2.4: Lysed bacterial cell.

Source: Prescott *et al.,* (2007).

These antibiotics disrupt cell wall synthesis. Thus as cells are formed and begin to generate a cell wall the presence of these antibiotics will result in weak and or deformed cell walls. Minor shifts in osmolarity can result in rupture of the cell. This group of antibiotics exerts little or no effect on bacteria that are not rapidly growing.The original penicillins were generally more effective against the gram-positive organism. They exerted little or no effect on gram-negative cells (the major exception to this was the effect that penicillin had on *Neisseria gonorrhea*, a gram-negative organism). In the case of most gram-negative organisms, the lack of activity by penicillin results from the barrier presented by the outer membrane of a gram-

negative cell and from differences in the enzymes involved in peptidoglycan synthesis. The semisynthetic penicillins that came later are able to penetrate this outer membrane and bind with the peptidoglycan forming enzymes of the gram-negative organism as well as those of the gram-positive organism. Ampicillin is slightly a modified form of penicillin but is one of the broader spectrum antibiotics on the market. Vancomycin interferes with the synthesis of the cell wall but is not a β-lactam antibiotic , its interference with the synthesis of peptidoglycan involves blocking the action of enzymes involved in the synthesis of peptidoglycan that are different than those blocked by the beta-lactam antimicrobials. Early preparations of this drug were contaminated with toxic materials that led to unwanted side effects (Prescott *et al.*, 2007).

2.6.1.1 Inhibition of Metabolic Pathways

Several classes of drugs inhibit the ability of the cell to synthesize the nucleotides needed in the synthesis of nucleic acids. This is accomplished by binding to the active sites of enzymes in the pathways involved in the production of these biochemicals.

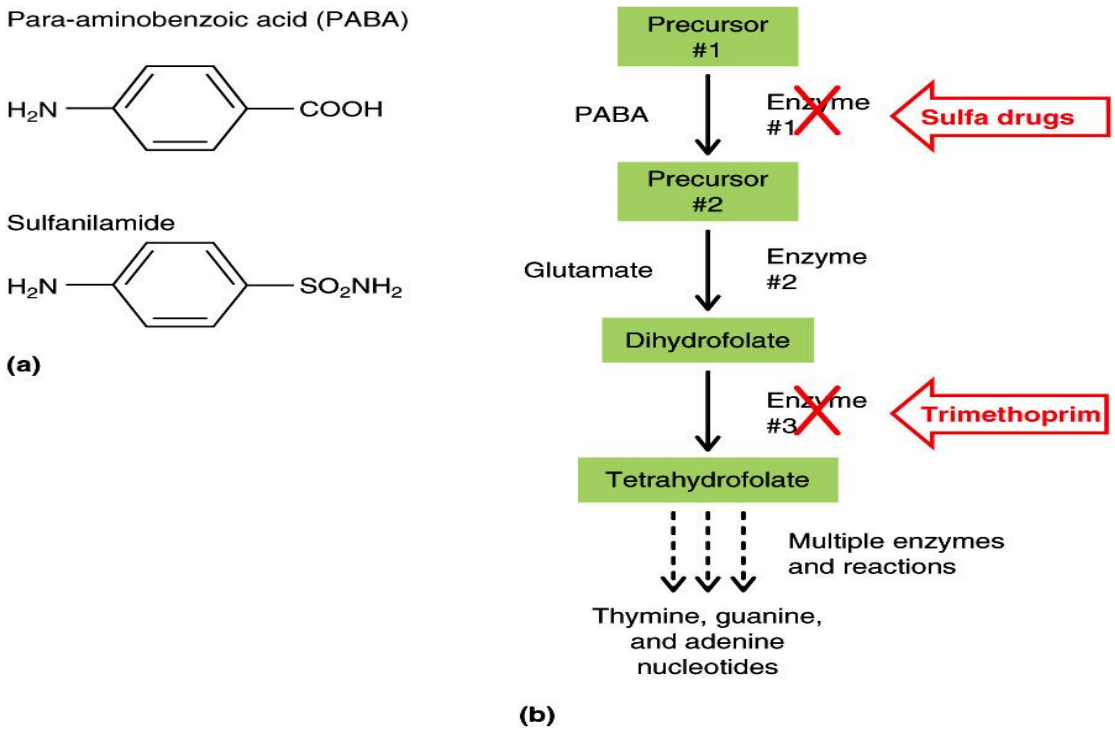


Fig2.5: Inhibition of folate pathway.

Source: Prescott *et al.,* (2007)

Once in the active site they bind and do not leave. Thus, the enzyme is rendered inactive. Sulfonamides (Sulfa drugs) inhibit the enzyme, which converts para-aminobenzoic acid (PABA) into dihydrofolic acid. Dihydrofolic acid eventually will become the nitrogenous bases known as adenine and guanine. Blocking PABA conversion to dihydrofolic acid effectively halts synthesis of nucleotides (Hugo and Russel, 2007).

2.6.1.2 Inhibition of Protein Synthesis

All cells must be able to carry on protein synthesis thus, all cells must have ribosomes. However, the ribosome of procaryotic cell differs slightly from that of the eukaryotic cell. This difference is the basis of action for several antibiotics, which

block translation in the procaryotic organism but have little or no effect on translation in the eukaryotic organism. These drugs include Tetracycline chloramphenicol, macrolides (erythromycin), aminoglycosides (streptomycin).

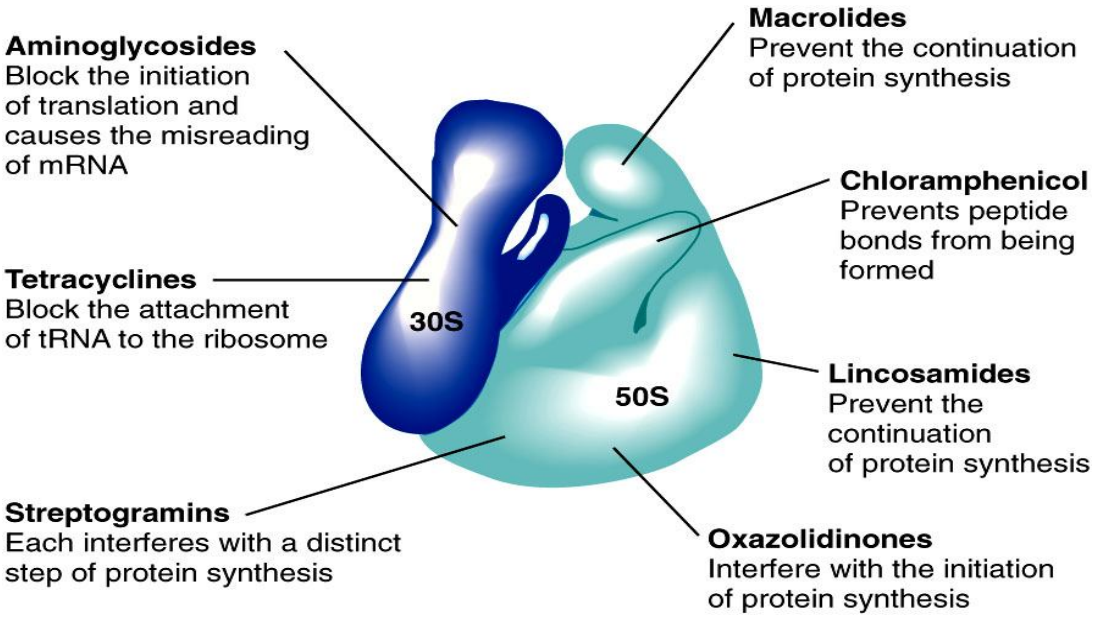


Fig 2.6: Antibacterial drugs that inhibit protein synthesis

Source: Nester *et al.,* (2004)

Aminoglycosides: Irreversibly binds to 30S ribosomal subunite, causing distortion and malfunction of ribosome, bocks initiation of translation and causes misreading of mRNA. Not effective against anaerobes, enterococci and streptococci. Often used in synergistic combination with β-lactam drugs, and allows aminoglycosides to enter cells that are often resistant (Hugo and Russel, 2007).

2.6.1.3 Inhibition of Cell Membrane Synthesis

The peptide antibiotics are produced like all other peptides, through the action of ribosomes, mRNA, etc. Their mode of action involves binding to phospholipids and disrupting the integrity of the cell membrane.

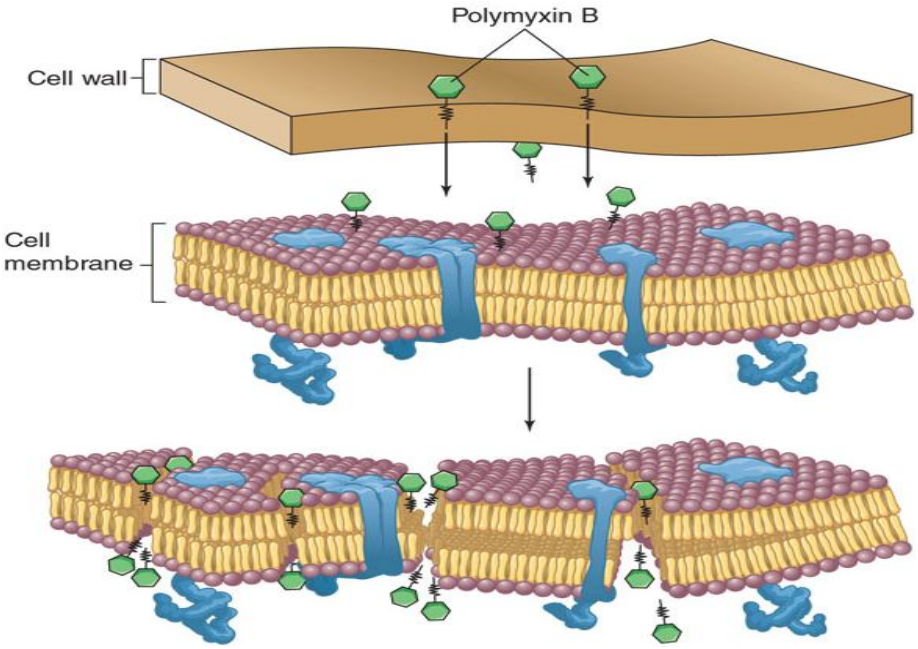


Fig 2.7: Antibacterial drug inhibiting cell membrane synthesis

Source: Nester *et al.,* (2004).

A few antibacterial damage bacterial membranes. Polymyxin B, bind to the membrane of gram-negative cell and alter the permeability, leading to leakage of cellular content and eventually death of the cell. These drug bind to eukaryotic cell, though to a lesser extent, which generally limit their use to tropical applications (Nester *et al.,* 2004).

2.6.1.4 Inhibition of Nucleic Acid Synthesis

The antibiotic rifampin (a derivative of the antibiotic rifamycin) inhibits the synthesis of mRNA. Differences in the RNA polymerase of procaryotic cells when compared with that of the eukaryotic cell allow this antimicrobial to selectively inhibit the action of procaryotic RNA polymerase, thus minimizing the toxicity of this drug. It is very effective in penetrating tissues and reaching the inside of the tubercles of tuberculosis. Consequently, it is used in controlling TB infections. Quinolones are a class of antibiotics that inhibit the function of DNA gyrase. In this way, they interfere with a cell's ability to carryout semiconservative replication. Levoquin is a commonly used quinolone (Hugo and Russel, 2007).

2.6.1.5 Inhibition of Lipoteichoic Acid.

Lipoteichoic acid is a major constituent of the cell wall of Gram-positive bacteria. It consists of teichoic acid, long chain of ribitol phosphate and anchored to the lipid bilayer via s glyceride. It act as regulator of autolytic enzymes(Muramidases), has an antigentic properties being able to stimulate specific immune response and is released from the bacterial cell mainly after bacteriolysis induced by lysozyme.It is presumed that synthesis of these secondary wall polymer might be essential for bacterial growth and might therefore serve as a target for antibiotic development. Daptomycin is an antibacterial drug that is active against MRSA, VRSA and VREF (Tally and Debruin, 2000). This antibiotic inhibit the formation of nucleotide-linked sugar-peptide precursors involved in peptidoglycan biosynthesis, this is because daptomyvin inhibit the accumulation of UDP-N-acetylmuramylpentapeptide (Nester *et al.*, 2004).

2.6.1.6 Inhibition of Aminoacyl- tRNA Synthetase

The aminoacyl-tRNA synthetase enzymes play crucial rolein protein biosyntheseis by cataylsing the synthesis of aminoacyl-tRNAs. Once this enzyme is inhibited, protein synthesis is halted, which result in the attenuation of bacterial growth under both invitro and infection condition (Yu *et al*., 2012). Mupirocin inhibit bacterial aminoacyl-tRNA synthetase by sharing structural homology with other antibiotic in clinical practice and consist of short fatty acid ester linked to monic acid, with the tail portion closely resembling the isoleucyl moiety of the isoleucyl-adenylate reaction intermediate (Yu *et al*., 2012).

2.6.1.7 Inhibition of Thymidylate Synthetase

Thymidylate synthetase is an enzyme used to generate deoxythymidine monophosphate (dTMP). This is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. By means of reductive methylation, deoxyuridine monophosphate (dUMP) and N5, N10-methylenetetrahydrofolate are together used to form dTMP, yielding dihydrofolate as secondary product (Daniel *et al*., 2003). Thymidylate synthase inhibitors are chemical agents that inhibit the enzyme thymidylate synthase and have potential as an anticancer chemotherapy. Fluorouracil is an example of such inhibitor. Raltitrexed works by inhibiting dihydrofolate reductase, an enzyme used in the synthesis oftetrahydrofolate,thereby prevent the synthesisofthymidlate and fully active after polyglutamylation, by inhibiting the formation of precursor pyrimidine nucleotides and preventing the formation of DNA and RNA required for growth and survival of both normal and cancer cells (Daniel *et al*., 2003).

2.6.1.8 Inhibition of DNA Helicases

DNA helicases are enzymes involved in the nucleoside triphosphate dependent unwinding of duplex DNA into single strands, an essential activity for replication. Replicative helicases require assistance from helicase loader to assemble at chromosomal origins of replication. Triaminotriazine I is a low micro molar inhibitor of the enzyme (Goldberg *et al.,* 2005)

2.6.1.9 Inhibition of Peptide Deformylase

The bacterial peptide deformylase is an enzyme responsible for catalyzing the removal of the N-formyl group from N- terminal methionine following translation. This enzyme is encoded by the defgene, which is present inall pathogenic bacteria, including Mycoplasma and Chlamydia species and which does not share a functionally equivalent gene in mammalian cells. Def gene is an essential gene in bacterial growth and survival (Chang *et al.,* 2003). Blockade of bacterial peptide deformylase produces inhibition of protein synthesis similar to mechanism of tetracycline, macrolide, lincosamides, chloramphenicol and streptogramins.Atinonin and macrolactin are the only naturally occurring peptide deformylase (Chan *et al.,* 2000; Yeo *et al.,* 2006).

2.6.2 Antibiotic Used in the Study

Cefunat (Cefuroxime Axetil) tablet United State Pharmacopoeia (USP) contain cefuroxime. It is a semi-synthetic and broad spectrum cephalosporin antibiotic for oral administration. Chemically, Cefuroxime axetil systematic IUPAC 1-Acetoxyethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-{[(2*Z*)-2-(2-furyl)-2-(methoxyimino)acetyl] amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. Its molecular formula is $C_{20}H_{22}N_4O_{10}S$ and has a molecular weight of 510.48 (Overington *et al*.,

2006). Cefuroxime Axetil is in the amorphous form and has the following structural formula.

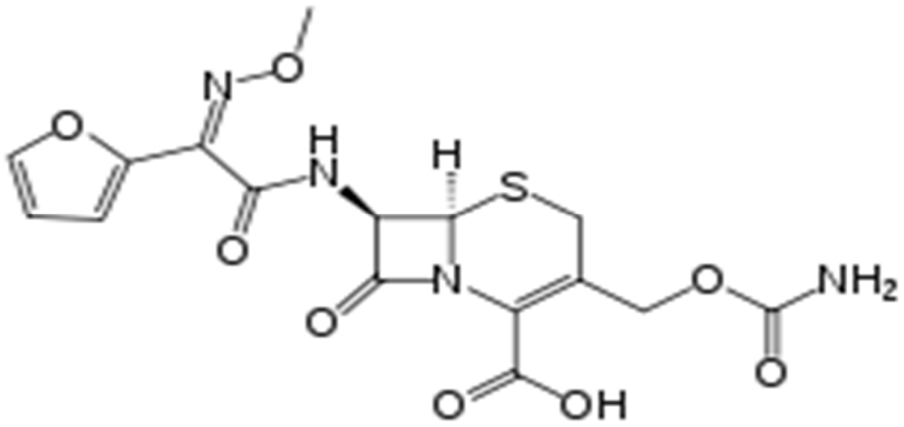


Figure 8: Chemical structure of Cefuroxime Axetil

(Prescott *et al.,* 2007).

2.6.2.1 Mechanism of Action

Cefuroxime is a β-lactam-type antibiotic, it is a second generation cephalosporin. Cephalosporins work the same way penecillins. It interfere with the peptidoglycan synthesis of the bacterial cell wall by inhibiting the final transpeptidation needed for the cross links. It binds to specific penecillins-binding-proteins (PBPs) located inside the bacterial cell wall, and inhibited third and last stage in bacterial cell wall. Therefore, it has bactericidal activity against a wide range of common pathogens, including many β-lactamase producing strains (Yu *et al*., 2012).

Therapeutic uses: Cefuroxime Axetil is used against aerobic gram-positive microorganisms (*Staphlococccus aureus, Streptococcus pneumoniae and Streptococcus pyogenes*). Also against gram-negative microorganisms (*Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria meningitides, Neisseria gonorrhoeae, and Spirochetes*). Cefuroxime axetil is used to reduce the development of drug-resistant bacteria and maintain effectiveness of cefuroxime

drugs and to prevent or treat infections that are proven or strongly suspected to be caused by susceptible bacteria (Overington *et al*., 2006). Meningococcal infection is a fatal disease of medical emergency often diagnosed via lumbar puncture. Fluid management and blood transfusion are needed (Dossett, 2001; Onipede *et al*., 2009).

6.2.2.1 Antibiotics

An antibiotic (antibacterial) is a chemical or natural product that is capable of kills micro-organisms such as bacteria (bactericide) or slows (inhibits) the growth of bacteria (bacteriostatic) (von Nussbaum *et al*., 2006). The short-term treatment consists in the use of antibiotics in the already infected individuals including – penicillins, ampicillin, chloramphenicol, ceftriaxone, ceftazidime, cefotaxime, cefuroxime, ciprofloxacin, alavusanate + amoxicillin etc. however, some serotype strains of bacterial meningitides are inherently resistant to most of the available antibiotics and some even resist more than one antibiotic and become more virulence. Bacterial organisms often tend to alter their chromosome by insertion, deletion or substitution of one or more of the genomic nucleotides to be able to resist the antibiotics or they may modify the antibiotic-target site to hamper the binding of such drugs to effective concentrations via either reducing penetration or permeability of the drug (decreased uptake) or increasing the efflux pump of such drugs. Some of the organisms can even develop antibiotic inactivating enzymes (Onipede *et al*., 2009; .WHO, 2011; Nikaido and Pages, 2012).

6.2.2.2 Chemoprophylaxis

Chemoprophylaxis is the use of drugs to prevent disease or infection such as vaccine. A vaccine is a biological preparation that improves immunity to a particular disease. Therefore, vaccine typically contains agent that resembles a disease-causing micro-

organism, and is often from the weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these micro-organisms that it later encounters (Greenwood, 2006; WHO, 2011). It is a long-term meningococcal chemoprophylaxis consists of the use of vaccines to reduce the transmission of the pathogenic bacteria. Sometimes, however, the fear of autism (brain disorder), vaccine associated paralysis (VAP) as a result of post vaccinal attack on human neuronal polypeptides and / or lack of good access road to rural dwellers hampers effective distribution of such immunization. The available meningococcal vaccine is the meningococcal polysaccharide vaccines available in various forms: -

- the bivalent form (for groups A and C)
- trivalent form (for groups A, C and W135) and
- tetravalent form (for groups A, C, Y and W135) (Onipede *et al*., 2009).
- The polysaccharide group B vaccine has not been successfully developed because it tends to mimic the human neurologic tissue polysaccharide. However, a type of vaccine called OMP (outer membrane proteins) is presently used in Cuba, New Zealand and Norway for strain-specific control of group B serotypes (Greenwood, 2006; Joel, 2010; WHO, 2011).

Reports showed that some efforts were being made to develop broad spectrum vaccines that can protect against the various serotype strains including the serotype B meningococcus strain. Yet the progress is still in the pipeline intensively for its development, but up-to-date literature search has not show any effect to the research (Andrew and Maiden, 2001; Riodan, 2001; Greenwood, 2006).

2.7 Description and Origin of *Allium sativum*

A. sativum (AS) is a food-plant originating from central Asia, but now grown everywhere in the world. For instance, this medicinal plant with bulb-stem resembling that of onion is now grown in Northern Nigeria. Garlic is the common name while *Allium sativum* is the scientific name. It belong to family of Lilliaceae and locally called 'tafarnuwa' in Hausa, 'allubosa ayu' in Yoruba, 'allibasa ayo' in Ibo and 'allivasa ahono' in Ebira. It grows 2-3 feet in height and is annual or biennial. The name garlic is derived from the shape of the plant-leaf- 'gar' meaning spear; thus it is a spear-shaped leaf plant. Garlic bulb is available throughout the seasons and its flowers, leaves and bulbs are often used as culinary and so it is often cultivated as a cash crop. Garlic has high nutritional value and also highly valued in folkloric medicines all over the world. It has been found to contain active principles such as allicin, allistatin, ajocine, allitridium, allixin, garlicin and scordinin which are of use in certain illnesses and conditions (Derrida, 2003; USDA, 2010). *A. sativum* also contains sulphorous volatile oil, macrophytonutrients, vitamin K, flavonoids, terpenes and iodoform compounds (Sachin, 2014). The *A. sativum* oil contains nigellone and eugenol often used for bronchial spasms (Durak *et al.*, 2004).



Plate 1: Garlic Plant in its Natural Habitat (Source: www.google.com/search/garlic+plant/)

Scientific name: *Allium sativum* Linn

Family: Lilliaceae

Common name: Garlic

Hausa name: *Tafarnuwa*

Yoruba name: *Allubosa ayu*

Igbo name: *Allibasa ayo*

Ebira name: *Allivasa ahono*

Arab: *Thuum*, Hindi: *Lahasun*, Philippino: *Bawang*.

2.8 Folkloric and Ethnomedical Uses

A. sativum has an ancient use as antiseptic, antibacterial, carminative, bleeding of the nose, diaphoretic, anthelmintic, antimicrobial, antiflatulence, and analgesic. It is also used locally for confluent smallpox, typhoid fever, dropsy or congestive heart failure, pulmonary tuberculosis or phthisis and for eye and ear infections (Derrida, 2003; WHO, 2010). It has also been reported to have antitumour and anticholesterolaemia properties whereby it is used to decrease low density lipoprotein and / or increase high density lipoprotein as to lower blood pressure. In China and Arabia, it has a history of

use as energizer or palliative and it is believed to have a natural nutritional healing power for cough, hay fever, pile or dysentery and also to promote digestion (dyspepsia) (WHO, 2010; Oduye, 2011).

2.9 Some Scientific Validations of Use of *Allium sativum*

Bostros *et al.* (2005) studied the antiparasitic properties of the methanolic extract of *A. sativum* bulbs against *Schistosoma mansoni* adult worm *in-vitro* studies. Their findings revealed strong biocidal effects against all stages of the parasite as it inhibited even the eggs laid by the adult female worms.

In 2012, Sen *et al*., investigated a similar anti-trematodal activity of the fresh extract of *A. sativum* bulbs against ruminant fluke in sheep and also revealed a high efficacy. It has also been evaluated for antibacterial and antifungal activities and strong heart remedy (Pekthong, 2008). In another experimental research studies, extracts of garlic bulbs showed protection against dyspepsia and cancer and also boosted the immume system (Durak, 2004). The organic acids in garlic had been noted to have immune modulating effects - stimulating cellular, humoral and innate immunities as well as preventing invasion of pathogenic organism and autoimmune diseases (Oduye, 2011). Garlic products are now commercially encapsulated and sold as dietary supplements for various ailments as hypercholesterolemia, high blood pressure, diabetes etc (Durak *et al*., 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Equipments and Instruments

Spectronic 21D/flame photometer (Unicam 969 AAS; Milton Roy Co., Rochester, New York)

Fast Sequential Atomic Absorption Spectrometer (Varian AA240FS; Amalgamated Biotech, USA)

Spectrophotometer (Buck 205 AA; Amersham Biosciences, USA)

Bacteria incubator (Dapco Model 630, USA)

Autoclave (Adelphi Co., USA)

Oven (Tatlock, 414462/27, UK)

Water bath (Gallenkamp, UK)

Electronic Weighing Balance (Mettler P162, UK) – (used for weighing extracts)

Electronic Weighing Balance (Ohaus Champ 11, UK) – (used for weighing animals)

3.2 Drugs, Solvents and Other Materials

Cefuroxime axetil (Cefunat, USP; BE207, Evans Medical Plc Lagos, Nigeria)

Ethanol (96%) (Sigma-Aldrich, USA).

Povidone Iodine (5%) (Betadine, USP; 2218, Jawa International Plc Lagos, Nigeria)

Mueller-Hinton Agar Media (Liofilchem, Italy)

Nutrient Broth Media (Maharashtra, India)

Nitric acid (Sigma-Aldrich, USA)

Hydrochloric acid (Sigma-Aldrich, USA)

Tween 80 (Sigma-Aldrich, USA)

Petri-dishes, Test-tubes, racks, forceps, wire loop, bend glass rod, filter paper, Stop watch, Mortar and pestle, electric blender, beaker, measuring cylinder.

3.3 Collection and Identification of Plant Material

The bulbs of *A. sativum* were obtained as fresh farm products from farmers at Sabon-Gari, Zaria, in Kaduna State, Nigeria in November, 2012. The plant's identification was authenticated by Mallam Umar S. Gallah at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and compared with voucher specimen number of 2156 for future reference.

3.4 Extraction of Plant Material

The collected bulbs of *A. sativum* (600 g) were washed and air dried under shade for 2 hours and the dry scaly outer covering was peeled-off to obtain the fresh garlic cloves which were then divided into three parts of 200 g each. These three portions were crushed separately for cold extraction according to the method of Fattouch *et al*., (2007). The first portion was homogenized and poured into a muslin cloth to squeeze out the juice, while second and third portions were homogenized and submerged into 500 ml of 96% ethanol and 500 ml of distilled water respectively for 24 hours and both filtered after thorough shaking. The first and second portions were freeze dried,

while the third portion was evaporated over water bath at 50^{o}C to obtain the powdered yield. The three samples obtained were then stored in separately labeled air-tight container for later use. The percentage (%) extract yield was calculated as follows:

% yield = weight of extract (g) / weight of garlic cloves (200 g) x 100

3.5 Solutions

Working concentrations to be used for the study were prepared from the obtained extracts (JEAS, EEAS and AEAS) using distilled water as a diluent. The required various concentrations to be used for each experiment were always freshly prepared from the stock solution of the extracts.

3.6 Test Clinical Bacteria Isolates

Five pathogenic clinical isolates (three anaerobic organisms): *Neisseria meningitides, Streptococus pneumoniae, Haemophilus influenzae* and (two aerobic organisms) *Klebsiella pneumoniae* and *Escherichia coli* obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria in Kaduna state, Nigeria were cultured in Mueller Hinton Agar (MHA) to obtain colonies of the organisms as described by Cheesebrough (2005) and Sharma and Aneja (2012).

3.7 Laboratory Animals

Male and female adult wistar rats weighing (171 – 179 g), in-bred in the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria were used. The animals

were fed with a standard diet and tap water *ad libitum* and kept at room temperature and were handled according to standard protocols for the use of laboratory animals. The studies were conducted according to the ethical guidelines and good laboratory practice for animal care and use in Ahmadu Bello University, Zaria (ABUZ-Animal Use and Care Policy, 2010).

3.8 Phytochemical Screening of *Allium sativum*

The methods of Trease and Evans (2002) and Sofowora (2008) were used to screen for the presence of various phytochemical constituents in the *A. sativum* extracts. Phytochemical constituents screened include alkaloids, anthraquinones, cardiac glycosides, carbohydrates, fat and oils, flavonoids, saponins, steroids, terpenoids and tannins. 2.0g of powders of each of the three extracts were dissolved in 100ml of distilled water for this screening experiment.

The tests carried out include:

i. **Mayer's test**: extract solution (1 ml) + 1 ml of Mayer's reagent added drop by drop that gives cream color precipitate indicates the presence of alkaloids.
ii. **Frothing test**: extract solution (2 ml) + 5 ml distilled water shaken vigorously; was observed for 15 minutes for a honey comb froth formation that indicates presence of saponins.
iii. **Shinoda test**: extract (0.1g) + 2ml of 5% ethanol + few magnesium chips + few drops of HCl; that forms a pink or red colour indicates the presence of flavonoids.

iv. **Molish test**: Five drops of Molish reagent + 2 ml of extract solution + 2 ml of concentrated H_2SO_4; that gives reddish ring indicate the presence of carbohydrates.

v. **Keller-Killiani test**: glacial acetic acid (2 ml) + 1 drop of $FeCl_3$ + 1 ml of extract solution + 2 ml of concentrated H_2SO_4; that gives a purple-brown ring indicate presence of cardiac glycosides.

vi. **Ferric chloride test**: extract solution (3) ml + 1 ml of 5% $FeCl_3$ (in 3 drops); that gives a deep-green color indicate the presence of tannins.

vii. **Borntrager's test**: extract solution (1 ml) + 2 ml of chloroform was shaken for 5 minutes and filtered, then filtrate + 10% NH_4OH; solution that gives a rose pink colour indicate the presence of anthraquinones.

viii. **Test for fats and oils**: Filter paper soaked in the extract solution or impregnated with extract was allowed to dry and checked for translucence films; that indicate the presence of fat and oils.

ix. **Liebermann-Buchard test**: extract (1 ml) + 1 ml of acetic acid anhydride was mixed gently + 1 ml of concentrated H_2SO_4; that gives a blue-green color indicates presence of steroids (ring) and terpenoids.

3.9 Analysis of Nutritional Composition and Proximate Values of *Allium sativum* Extract (JEAS)

Analytical automated instruments including spectronic 21D / flame photometer, unicam 969 AA spectrometer (AAS), fast sequential atomic spectrometer AA240FS and AA spectrophotometer Buck205 were used to analyze for both nutritional composition and their proximate values in the JEAS according to the methods described by Fahey (2005); Naturopathy (2010) and Van (2010). Distilled water,

concentrated nitric acid (HNO_3) and hydrochloric acid (HCl) were used to digest the extract, which was then heated in water bath at 90^{o}C and filtered to obtain the filtrate for the analytical studies for *A. sativum* nutritional composition and zeolite herbominerals. The presence of dietary phytonutrients including carbohydrates, proteins, dietary fibres, fats and oils, vitamins as well as the zeolite herbomineral elements and their proximate values were determined. 2.0g of JEAS powder was dissolved in 100ml of DH_2O for the quantitative determination of vitamins, macro- and micro-elements and other nutrients. 10ml for vitamin analysis (200mg), 30ml for macro/micro (zeolite) element analysis (600mg) and 30ml for other nutrients analysis (600mg) respectively.

3.10 Determination of Blood Clotting Time (Antihaemorrhagic) Effect of *Allium sativum* Extracts

The concentrations of the three extracts used throughout the studies were deduced from the pilot studies for both antihaemorrhagic effects and antibacterial activities. This experiment was carried out according to Mielke's method described by Mark *et al*., (2005) with standard rat blood clotting time of 2-5 minutes. Twelve adult wistar rats of both sexes were used to determine the effect of the three extracts of *A. sativum* under investigation (JEAS, EEAS and AEAS) on the blood clotting time shortening. The animals were divided into 4 groups of 3 rats per group as control, JEAS, EEAS and AEAS. Sterilized scalpel blades were used to lacerate wounds of about 1mm deep and 2 mm long below the groins (thighs) of the hind legs of the rats in all the groups using one hind leg each in two alternate days. Thus, both hind legs of the three rats in each group were used to obtain a total of six experiments per group (n = 6). The 4 groups wounded rats were then given an immediate treatment by topical

application of standard antiseptic microbicidal astringent (5%Wosan Jawa Povidone Iodine), 5 mg/ml of JEAS, 5 mg/ml of EEAS and 5 mg/ml of AEAS respectively and observed for clotting time (the time bleeding stops). The mean blood clotting time for the 3 rats in each group for the duplicate experiments were then calculated and compared with the control group.

3.11 Determination of Antibacterial Activity of *Allium sativum* Extracts

The bacterial cultures were prepared by transferring with sterile wire loop, each bacterium cell into a Nutrient Broth Medium which was incubated at 37^{o}C for 24 hours as described by WHO-BS (2011) to obtain a colony of the bacterial cells. The cells were maintained in Nutrient Broth Media in universal bottles labeled for each bacterium. Three tubes (1, 2, 3) each containing 9 ml of normal saline were set up for 3-fold serial dilution (1:1000) for the gram-positive bacterium, in which 1 ml of the overnight culture was transferred into the first test tube and mixed; and 1ml of this then taken into the second test tube from which another 1ml was removed into the third tube which was then deemed to be 1x10^{3} colony forming unit / ml (CFU/ml). The same serial dilution, but in a fourth tube containing 4.5 ml of normal saline and 4-fold dilution (1:5000) was also performed for the gram-negative bacteria to the last concentration of 1x10^{5}CFU/ml. The last diluted concentrations (5 test tubes of the bacterial organisms) were incubated at 37^{o}C for 24 hours after which, the bacterial organisms (suspension) were inoculated on prepared Molten sterile Mueller-Hinton agar plates by taking 2 ml of each of the organisms and flooding it over the agar surface by agar well diffusion method of Sharma and Aneja, (2012). Four different concentrations (10, 15, 20 and 25 mg/ml) and 5 mg/ml of the standard drug (cefuroxime) were used to study the growth inhibitory effect for each of the three

extracts of *A. sativum*. Wells were bored on the agar dishes and a drop of the molten agar was used to seal the bottoms of the bored wells prior to filling them with 0.2 ml of each the various drug concentrations. The plates were then kept for 1 hour to diffuse and then incubated at 37°C for 24 hours. The experiment was performed in duplicate and the zones of growth inhibition around the two wells for each drug concentrations were measured in millimeter using a ruler. The mean of the duplicate experiments for each concentration of the extracts were then calculated and recorded as the growth inhibitory zone of the extract concentrations for each of the organisms (Cheesebrough, 2005; WHO-BS, 2011).

3.12 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The same extracts concentrations of 10, 15, 20 and 25 mg/ml of the three *A. sativum* were used for MIC and MBC experiments. Serial dilutions were made for each concentration as well as for the standard drug whereby ten test tubes with each containing 9 ml distilled water were set up for each concentration and 1 ml of each concentration taken into the first test tube and from which 1 ml was taken into the second test-tube and continuously like that until the last test tube after which the 1ml taken from it was discarded. Then 2 ml of each of these mixtures (varied concentrations) in test tubes were taken respectively into various petri-dishes containing 8 ml of molten agar that had been labeled for each diluted mixture and then mixed to form broth dilution. Similar dilution was carried out for each of the three extracts and in all; a hundred and fifty mixture petri-dishes were gotten (50 for JEAS, 50 for EEAS and 50 for AEAS). All of these were kept for 2 hours to solidify after which a sterile forcep was used to place 5 sterile filter paper discs of 6 mm in

diameter on each solidified agar plate surface. A micropipette was used to inoculate 0.02 ml of each of the five bacterial isolates on each of the discs and this was done for all the four concentrations of the three extracts under study and also for the standard drug. The plates were then incubated at 37^0C for 24hrs. The lowest diluted concentration of each extract stock concentration in which there was no visible growth of any of the bacterial organisms was considered as the Minimum Inhibitory Concentration (MIC) for the organism in question (Cheesebrough, 2005). The Minimum Bactericidal Concentration (MBC) of the various extracts were performed from the lowest concentration (MIC) of the dilutions of the 4 stock concentrations and the higher concentrations above it. For instance, the 10 mg/ml has its diluted concentration of 2.5 mg/ml as its MIC and thus, three test tubes labeled as 2.5, 5.0 and 10 mg/ml and containing 2 ml nutrient broth and 3 drops of 3% v/v TW 80 were set for MBC. Then, filter paper discs of the bacterial organisms from the corresponding MIC experiment were transferred into the test tubes respectively and again incubated at 37^0C for 24 hours and checked for growth inhibition (Cheesebrough, 2005; Sharma and Aneja, 2012).

3.13 Statistical Analysis

Results obtained were recorded as mean ± SEM and subjected to one way analysis of variance (ANOVA). Where significant differences exist, Waller Duncan post hoc test was performed using Statistical Analysis System (SAS, software version 2002). The results were regarded as significant at $P< 0.05$ or lower.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction of the Plant Material

The calculated percentage yield from the 200 g of *Allium sativum* L bulbs used for the extraction was as given in table 4.1 below:

Table 4.1: Percentage Yield of *Allium sativum* Extracts

Extract	% Yield (w/w)	Observed Colouration
JEAS	12.7	light yellow powder
EEAS	17.2	light yellow powder
AEAS	14.7	Brown powder

4.2 Phytochemical Screening of the Extract

The table below is a summary of the phytochemical components or secondary metabolites of the extract. From the obtained result, anthraquinone and tannins were not present in the extract of the bulbs.

Table 4.2: The Phytochemical Constituents of *Allium sativum*(AS) Bulb Extracts (JEAS, EEAS and AEAS)

Phytochemical Components	Inference
Alkaloids	Present
Anthraquinone	Absent
Carbohydrates	Present
Cardiac glycosides	Present
Fats and Oils	Present
Flavonoids	Present
Saponins	Present
Steroidal ring	Present
Terpenoids	Present
Tannins	Absent

Note: JEAS= juice extract, EEAS= ethanolic extract and AEAS=aqueous extract

4.3 The Nutritional Composition and Proximate Values in the JEAS Extract

From the analysis carried out for nutritional composition, nutrients such as carbohydrate, dietary fibre, protein, fat, vitamins, macro- and micro-herbominerals with their proximate values were observed as summarized in Table 4.3.

Table 4.3: The Nutritional Composition and Proximate Values of Juice Extract of *Allium sativum* (JEAS)

Nutritional components	
Vitamins	**Proximate Values (mg)/200mg**
Vit A (β-Carotene)	14.00
Vit B_1 (Thiamine)	1.20
Vit B_2 (Riboflavin)	0.81
Vit B_3 (Niacin)	0.82
Vit B_5 (Pantothenic acid)	0.71
VitB_6 (Pyridoxine)	2.35
Vit B_9 (Folic acid)	0.50
Vit H (Biotin)	95.00
Vit B_{12} (Cyanocobalamin)	8.22
Vit C (Ascorbic acid)	21.40
Vit D (Ergocalciferol)	4.83
Vit E (α-Tocopherol)	31.20
Vit K (Phytonadione)	2.16
Macro/Micro (Zeolite) Elements	**Proximate Values (mg)/600mg**
Sodium	19.00
Potassium	154.00
Calcium	184.00
Magnesium	26.00
Phosphorus	390.00
Sulphur	450.00
Iron	1.82
Manganese	0.03
Silver	0.01
Iodine	0.05
Zinc	1.18
Selenium	0.014
Other Nutrients	**Proximate Values (mg)/600mg**
Carbohydrate	54.39
Dietary Fibre	2.20
Protein	32.40
Fat	4.00

4.4 The Blood Clotting Time Effect of *Allium sativum* Extracts in Rats

The result obtained showed that *A. sativum* extracts showed significant reduction ($p<0.05$) in bleeding time and shortened clot time at the site of the inflicted injuries on wistar rats compared with that of standard drug (control) (See Table 4.4).

Table 4.4: Effect of Blood Clotting Time of *A. sativum* Extracts in Wistar Rats' Wounds and Arranged According to Waller Duncan Ranking in Descending Order

Treatment	Clotting Time (minutes)
5% Povidone Iodine (control)	1.05± 0.01
5mg/ml JEAS	0.94± 0.01[b]
5mg/ml EEAS	0.99± 0.04[a]
5mg/ml AEAS	1.20± 0.04

Values are presented as Mean ± SEM, b and a = P< 0.05 compared to the standard drug (control) - One Way ANOVA followed by Waller Duncan Post Hoc Test, Df =3, N=6.

4.5 Inhibitory Effect of *Allium sativum* Extracts on Growth of Clinical Bacterial Isolates

The result obtained showed that *A. sativum* extract inhibited the growth of clinical bacterial isolates at 30mg/ml showed significant inhibitory $P<0.05$ in in-*vitro* susceptibility test compared with that of standard Drug (control) (See Table 4.5).

Table 4.5: Inhibitory Effect of *A. sativum* Extracts on the Growth of Clinical Bacterial Isolates

Test Exts.	Zone of growth inhibition (mm) of the extracts at drug concentrations (mg/ml)					Bacterial orgs
	10	15	20	25	5 (cefuroxime)	
JEAS	20.5±0.50	25.5±0.50	29.5±0.50	30.5±0.50[a]	35	*Streptococcus pneumoniae*
EEAS	-	-	-	12.5±0.50	35	
AEAS	-	-	-	-	35	
JEAS	28.0±0.00	33.5±0.50[a]	34.0±0.00[a]	36.0±0.00[a]	35	*Neisseria meningitides*
EEAS	-	-	14.5±0.50	17.5±0.50	35	
AEAS	-	-	-	-	35	
JEAS	-	-	-	-	20	*Klebsiella pneumoniae*
EEAS	-	-	-	-	20	
AEAS	-	-	-	-	20	
JEAS	23.0±0.00	26.5±0.50	28.0±0.00	30.5±0.50[a]	35	*Haemophilus influenzae*
EEAS	-	15.5±0.50	18.5±0.50	20.0±0.00	35	
AEAS	-	-	-	-	35	
JEAS	29.0±0.00	31.5±0.50[a]	32.0±0.00[a]	35.5±0.50[a]	35	*Escherichia coli*
EEAS	-	-	-	-	35	
AEAS	15.5±0.50	16.0±0.00	19.0±0.00	20.5±0.50	35	

- = No zone of inhibition; Values are presented as Mean ± SEM, a=$P < 0.05$ compared to the standard drug (control) - One way ANOVA followed by Waller Duncan Post Hoc Test, Df=4 and N=15.

4.6 Determination of Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC)

The result obtained shown below revealed the efficacies of *A. sativum* extracts' inhibitory effects, and the rate of killing the pathogenic bacteria isolates (Table 4.6). The time of killing of bacteria can easily be deduce to be the ratio of MBC/ MIC = 1 or closer to 1, the rate of killing (KR) will be rapid, and is the potential weapon for eradicating or combating pathogenic bacteria and thereby reduce hemorrhagic sepsis with systemic dysfunctions (Pilkhwal *et al*., 2010).

Table 4.6: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of Standard Drug (Cefuroxime axetil) (5mg/ml)

Clinical Isolates + Cefuroxime axetil	Concentration of standard drug (cefuroxime axetil) in (mg/ml)												
	5	2.5	1.25	0.63	0.313	0.157	0.76	0.04	0.02	0.01	MIC Values	MBC Values	MBC/MIC =KR
S. pneumoniae	-	-	-	-	-	-	-	-	+	+	0.04	0.04	1.00
N. meningitides	-	-	-	-	-	-	-	-	+	+	0.04	0.76	19.00
K. pneumoniae	-	-	-	-	-	-	+	+	+	+	0.157	0.63	4.01
H. influenzae	-	-	-	-	-	-	-	-	+	+	0.04	0.157	4.00
E. coli	-	-	-	-	-	-	-	-	-	+	0.02	0.04	2.00

- = No viable growth, + = viable growth

4.6.1 Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (10mg/ml)

Clinical Isolates + Extracts		Concentration of *A. sativum* extracts in (mg/ml)												
		10	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04	0.02	MIC Values	MBC Values	MBC/ MIC=KR
S. pneumoniae	JEAS	-	-	-	+	+	+	+	+	+	+	2.5	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	10	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	+	+	+	+	+	+	+	2.5	NB	
	EEAS		+	+	+	+	+	+	+	+	+	10	NB	
	AEAS	- +	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	+	+	+	+	+	+	+	+	5	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	+	+	+	+	+	0.63	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	+	+	+	+	+	+	+	+	5	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

4.6.2 Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (15mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (mg/ml)												
		15	7.5	3.8	1.88	0.94	0.47	0.23	0.12	0.06	0.03	MIC Values	MBC Values	MBC/ MIC=KR
S. pneumoniae	JEAS	-	-	-	-	+	+	+	+	+	+	1.88	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	15	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	-	-	+	+	+	+	+	0.94	NB	
	EEAS	-	+	+	+	+	+	+	+	+	+	15	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	-	+	+	+	+	+	+	+	3.8	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	-	-	+	+	+	0.23	NB	
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	-	+	+	+	+	+	+	+	3.8	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

4.6.3 Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (20mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (Mg/ml)												
		20	10	5	2.5	1.25	0.63	0.313	0.16	0.08	0.04	MIC Values	MBC Values	MBC/ MIC=KR
S. pneumoniae	JEAS	-	-	-	-	-	+	+	+	+	+	1.25	2.5	2.00
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	-	-	-	+	+	+	+	0.63	1.25	2.00
	EEAS	-	+	+	+	+	+	+	+	+	+	20	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	-	-	+	+	+	+	+	+	2.5	5.0	2.00
	EEAS	-	-	-	+	+	+	+	+	+	+	5	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	-	-	-	+	+	0.16	0.313	2.00
	EEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
	AEAS	-	-	-	-	+	+	+	+	+	+	2.5	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

4.6.4 Minimum inhibitory and Minimum Bactericidal Concentrations (MIC & MBC) of the Bulb Extracts (Juice Extract, Ethanolic Extract and Aqueous Extract) of *A. sativum* (25mg/ml)

Clinical Isolates + Extracts		Concentration of *Allium sativum* extracts in (mg/ml)												
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	MIC Values	MBC Values	MBC/ MIC=KR
S. Pneumoniae	JEAS	-	-	-	-	-	-	+	+	+	+	0.78	1.56	2.00
	EEAS	-	+	+	+	+	+	+	+	+	+	25	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
N. meningitides	JEAS	-	-	-	-	-	-	-	+	+	+	0.39	0.78	2.00
	EEAS	-	+	+	+	+	+	+	+	+	+	25	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
K. pneumoniae	JEAS											NA	NA	
	EEAS											NA	NA	
	AEAS											NA	NA	
H. influenzae	JEAS	-	-	-	-	-	+	+	+	+	+	1.56	3.13	2.00
	EEAS	-	-	-	-	+	+	+	+	+	+	3.13	NB	
	AEAS	+	+	+	+	+	+	+	+	+	+	NA	NA	
E. coli	JEAS	-	-	-	-	-	-	-	-	-	+	0.10	0.20	2.00
	EEAS	-	-	-	-	-	+	+	+	+	+	1.56	NB	
	AEAS	-	-	-	-	-	-	-	+	+	+	0.39	NB	

- = No viable growth, + = Viable growth, NA=No activity, NB= Non Bactericidal

CHAPTER FIVE

5.0 DISCUSSION

Allium sativum extracts were found to express high antibacterial activities on *Escherichia coli*, *Helicobacter pyroli*, *Salmonella hadar, Neisseria meningitides, Streptococcus pnumoniae, Salmonella typhi* and *Enterobacteriacaea*, in which *E. coli* and *Salmonella typhi* are the most common of gastrointestinal infections (Derrida, 2003; Fattouch *et al*., 2007). About 80% of the human populations in Nigeria are long-time carriers of these bacterial species which are highly virulent in neonates, growing infants and young adults (Cuevas *et al*., 2007; Okoko *et al*., 2009), and are responsible for infections like ulcer, dysentery, septicaemia and typhoid fever. These Gram-negative bacteria can also cause life-threatening diseases such as otitis media, pneumonia, bacterial meningitis, endocarditis, chest pain, respiratory distress and bacterial hemorrhagic sepsis (Onipede *et al*., 2009; Hackam *et al*., 2011).

The aqueous extract of *A. sativum* was evaporated over a water bath at 50^0C to obtain AEAS powder, which was brown in colour and had low biologic activities. It may be due to heat applied via water bath and this led to loss of some of its bioactive constituents that heat labile. Similar activity was observed and reported by Greiner and Konielzny (2006). JEAS powder and EEAS powder showed significant high biologic activities. Different solvents yielded substantial differences in the proportion of extracts obtained. The differences in the percentage yields might be due to the polarity of the solvents, the less polar solvents such as ethanol, yielding greater quantities of the extracts compared with the more polar solvents such as water (Table 4.1). JEAS was the least in percentage yield but more potent and efficacious in biologic activities than EEAS and AEAS (Table 4.4; Table 4.5 and Table 4.6).

The phytochemical screening of the bulb extracts (JEAS, EEAS and AEAS) for bioactive constituents observed in *A. sativum* extracts revealed phytoconstituents with biological activities (Table 4.2). Some of these compounds are responsible for the characteristic odours like allylmethylsulphate (AMS) as garlicophobia, pungencies and colour of the plants while others give a particular plant its culinary, medicinal or poisonous virtue (Trease and Evans, 2002; Sofowora, 2008). From the result of the phytochemical screening the extracts were found to contain saponins, fats and oils, flavonoids, alkaloids, carbohydrates, cardiac glycosides and terpenoids. In previous studies that were also carried out, the same phytochemical constituents were reported to possess antimicrobial activities and effects on heart (Derrida, 2003; Pekthong, 2008). The secondary metabolites have been variously reported to possess appreciable inhibitory activities against various organisms such as bacteria, fungi, parasites and viruses; flavonoids and sulphorous volatile oils have been reported to be synthesized by plant in response to microbial infection and have been shown to have antibacterial activities (Shyh-Ming and Mei-Chin, 2001; Derrida, 2003). The antihemorrhagic and antibacterial properties of these extracts can be linked to the presence of alkaloids, cardiac glycosides, carbohydrates, fats and oils, flavonoids, saponins, terpenoids, vitamin K and iodine. Indeed, members of these phytoconstituents are known to possess antihemorrhagic and antibacterial activities coupled with primary metabolites (vitamins and antioxidants) and also zeolite herbominerals (Fahey, 2005; Larguita and Gemma, 2008; Yoshiriro and Hajime, 2012). Alkaloids, saponins, cardiac glycosides, oils, fats and terpenoids were also reported to have demonstrated activity against bacteria; have antidropsy effect and reduced inflammo-pathologic responses in the systemic and central nervous system (Derrida, 2003; Oduye, 2011). Alkaloid, saponins, volatile sulphorous oil and flavonoids in this plant are known to form

complexes with peptidoglycans, sterols and other cell wall components of bacteria resulting to cell leakage, cellular apoptosis and finally death of bacteria (Fattouch *et al.,* 2007; Pilkhwal *et al*., 2010).

Since the crude juice, ethanol and aqueous bulb extracts of *A. sativum* eradicated *Streptococcus pneumoniae, Neisseria meningitides, Haemophilus influenzae* and *Escherichia coli* in this *in vitro* antibacterial studies and also showed antihemorrhagic effect, the extracts could be used in the treatment of bacterial meningitis. Shyh-Ming and Mei-Chin (2001) and Fahey (2005) attributed the effectiveness of the fresh crude juice of *A. sativum* in the treatment of bacterial disease to the combined action (phytosynergistic effect) of these secondary metabolites as well as primary metabolites. There have been several reports on the naturally occurring phytoconstituents found in this plant such as steroids and cardiac glycosides indicating their usefulness in the treatment of diseases associated with the heart and dropsy (Trease and Evans, 2002). Presence of saponin, oil, terpenoids, alkaloids and flavonoids in all the extracts (Table 4.2) suggested their ability to play such role as antibacterial, antihaemorrhagic agents and also as antioxidants present in vit. A, vit. C, vit. E and vit. H. A similar study has reported these phytoconstituents as having potential antimicrobial and coagulative (shorter blood clotting time) properties e.g wellawel (*Chromolaena odorata*) which showed promising antimicrobial and coagulative activities (Larguita and Gemma, 2008). The presence of vitamin K, oils, iodine and saponins was also reported to hasten the healing of wounds and inflamed vasogenic injuries (Oduye, 2011; Sachin, 2014). Saponin has been reported to have effects on blood vessel spasm at site of vasogenic injury, and possess beneficial properties of lowering cholesterol level in cardiovascular system of the body (Durak *et al*., 2004).

Table 4.3 shows the nutritional composition and proximate values of the JEAS of *A. sativum.* The result showed the presence of carbohydrates, dietary fibres, proteins, fats, vitamins and macro- and micro-elements (zeolite herbominerals) as primary metabolites. Thus, this plant extract contained all classes of food nutrients, a complete nutritious and balanced diet as nutritional utility plays a vital role in biocidal or bactericidal effect on these pathogenic bacteria. In fact, the presence of all food nutrients in *A. sativum* bulb extract is an indication that it contained a natural vital nutritional source for health maintenance, blood interactor; prevents and boosts the body immune system against diseases. Both the primary and secondary metabolites of this plant, work best in combination by supporting each other as integrative medicines (Fahey, 2005; Oduye, 2011; Sachin, 2014). In addition, nutritional and medicinal properties of *A. sativum* extract is said to be pharmacologically active through zeolite-chelating, antioxidant and ameliorative properties. The findings observed were antioxidant properties, chelation effects and nanopharmacologic of Silver, Manganese, Zinc, Iron and Selenium; which exerts biocidal properties as well as stimulates immune system to cushioning the challenges of the BM pathogens, its potency of this plant is engineered by nature (Greiner and Konielzny, 2006; Walter, 2011; Sachin, 2014). However, *A. cepa* has been reported to have antibacterial and antihaemorrhagic activities which were found to be due to the presence of saponins, flavonoids, alkaloids, vitamins, oils and iodine (Oduye, 2011; Sachin, 2014). The antibacterial and antihaemorrhagic properties of *A. sativum* extracts may be due to these phytoconstituents since they were found in *A. sativum* and immuno-pharmacologic effects on immune system to activate, or manipulate the immune system and rapid wound healing.

The presence in *A. sativum* of phytoconstituents, which have medicinal properties, dietary supplements and food ingredients (Table 4.2 and Table 4.3) were demonstrated to possess health benefits and are used as combined tools for treating bacterial meningitis. Its zeolite herbominerals such as silver ions, zinc ions, selenium ions have bactericidal effect on bacterial meningitis pathogens through their nanopharmacologic and chelation therapy (Walter, 2011). It protects entire body by stimulating the immune system, prevents infectious diseases, improves health, and supports the structures (collagens) (Fahey, 2005; Van, 2010; Oduye, 2011).

Table 4.4 showed the blood clotting effect of *A. sativum* extracts which were evaluated by mean blood clotting time in minutes. The afflicted wounds on rats of the tested groups were treated immediately with 5 mg/ml of extracts (JEAS, EEAS and AEAS) while the control group rats with 5% Povidone Iodine as standard drug. *A. sativum* extracts have been found to contain steroidal saponins, terpenoids, fats and sulphorous oils and flavonoids. A similar study of Onion (*Allium cepa*) was shown to contain such phytoconstituents as volatile oils, saponins, vitamin K, iodine and tannins and had demonstrated blood clotting reduction time (Sachin, 2014). The blood clotting time of JEAS extract showed better response with least mean of blood clotting time (0.94± 0.01) followed by EEAS (0.99±0.04). These results were statistically significant ($p<0.05$) positive effects when compared with standard drug (1.05±0.01) used. The hemostatic effects of *A. sativum* may bring about the binding of the damaged tissues together and stops bleeding at site of vasogenic injury. This antihemorrhagic study suggests that fats and oils in these plant extracts (Table 4.2) stabilize various mucosal membranes around the damaged tissues (Sachin, 2014). The mechanism may be by vascular spasm and blood lipid interaction as well as providing increased biochemical signals resulting in blood clot. There will be fibrinogen

formation (liquid) around the damaged tissues, phospholipids, phospholecithins, phosphatidylcholine as contributing factors and thrombin activation. It activated thrombin, then act on fibrinogen to form fibrin (a mass solid), then to form jelly-like meshwork of cross-linked fibrin polymer (solid) that plug the gap and ceases bleeding (Corey *et al*., 2011). The hemostatic effect of this plant extract has scientifically supports the local usage of this plant in treating bacterial meningitis and associated hemorrhagic sepsis.

A. sativum extracts were found to possess higher antibacterial activities against *Streptococcus pneumoniae, Neisseria meningitides, Haemophilus influenzae, Escherichia coli* but not *Klebsiella pneumoniae* (Table 4.5 and Table 4.6). *Klebsiella pneumoniae* was highly resistant to the four concentrations of the three extracts used 10, 15, 20, 25 mg/ml respectively (Table 4.5). The antibacterial effects of the *A. sativum* extracts were measured using diameter zone of growth of inhibition, with a measurement greater than 10 mm indicating good antibacterial activity (Cheesebrough, 2005).

JEAS extract showed more antibacterial activity than EEAS and AEAS extracts, the difference in their antibacterial susceptibilities might be attributed to the phytoconstituents present in juice extract which is free from other solvents in nature. On the other hand, this might be due to solvent interaction with the extract phytoconstituents and probably hot steam applied may have contributed to their low biologic activity compared to JEAS, and their mean antibacterial activity were statistically significant ($p<0.05$) positive effects. Shyh-Ming and Mei-Chin (2001) and Fattouch *et al*., (2007) also reported similar properties in their findings and observed similar activities against pathogenic bacteria.

This study demonstrated that different extracts exhibited different levels of activities, which were also dependent on the nature of the test organisms. Generally, the bacteria isolates tested in this work showed susceptibilities to the extracts except *Klebsiella pneumoniae* (Gram-negative bacterium) which was highly resistant to the three extracts (Table 4.5 and Table 4.6). The zone of inhibition against *Neisseria meningitides* was 36 mm for JEAS extract while it is 30 mm against *Streptococcus pneumoniae* (Table 4.5). *Neisseria meningitides* accounts for about 80% of bacterial meningitis cases (Sultan *et al*., 2005).

Pharmacologically, the JEAS extract has demonstrated an interesting pattern of bacterial susceptibility against clinical isolates tested. Its MBC values were higher than the MIC values, indicating that the bactericidal effect of the JEAS could result to bacterial eradication. For instance, the MICs and MBCs of the JEAS against *S. pneumoniae* were 0.78 mg/ml and 1.56 mg/ml and MBC/MIC= 2 while *N. meningitides* were 0.39 mg/ml and 0.78 mg/ml from concentration of 25mg/ml and MBC/MIC= 2 compared with the reference standard drug. Whose *N. meningitides* MIC and MBC was 0.76 mg/ml and 0.04 mg/ml and MBC/MIC=19. It was reported that the bacterial killing rate of an extract whose ratio of MBC to MIC is closer to one (1) rate of time, and will be rapid and a potential weapon against pathogenic bacteria eradication (Pilkhwal *et al*., 2010).

The differences in the susceptibility of the clinical isolates to the plant extracts can be related to the cell wall composition of the bacterial organisms. In general, Gram-positive bacteria have a cell wall of lipoteichoic acid and muramyldipeptide which are more susceptible to antibacterial agents than Gram-negative bacteria. The Gram negative bacteria have their cell wall surrounded by lipopolysaccharides and arabinoside peptidoglycan, which prevent penetration of antibiotics by efflux pump

through their cell wall. *N. meningitides* was more susceptible to the extracts especially the JEAS at a very low concentration of 0.78 mg/ml (Table 4.5). This is of great importance as it was reported that these organisms have developed resistance to many synthetic antibiotics, which increase cost of effective treatment in clinical management of bacterial meningitis (Sindhu *et al*., 2011). Again, high activity of any antibacterial agent at low concentration is very essential for chemotherapeutic purposes to minimise toxicity in the patient system (Surbhi *et al*., 2011).

Generally, the degree of the test plant extracts antibacterial activity varies from test organisms to another, thus large zones of inhibition were produced by the sensitive organisms compared to the resistant isolates. However, these activities are concentration dependent, irrespective of the test organism involved. This variation in level of the activity among the extracts could be due to the difference in solubility of the active ingredient in each solvent on one hand and to the constitutional or structural variability of the tested organisms on the other hand. Generally, JEAS had a lower MIC values (0.10 mg/ml - 5 mg/ml) and MBC values (0.20 mg/ml - 20 mg/ml) against bacteria isolates compared with EEAS and AEAS, which was similar to the findings observed and reported by Fattouch *et al.,* (2007). When biocidal concentrations were considered, *A. sativum* extract (JEAS) MBC was relatively higher than the standard antibiotic agent investigated in this study (Table 4.6).

Table 4.6.0 showed bactericidal activity of JEAS and cefuroxime which were statistically significant ($p<0.05$) against the clinical isolates. The bactericidal activity of the test extracts (JEAS, EEAS and AEAS) were rapid from the onset and generally based on a concentration dependent manner. It is a concentration dependent activity which was exhibited by the extracts against these clinical pathogenic organisms, an increase in concentration gave rise to an increase in the diameter zone of growth inhibition. Rate of killing bacteria (MBC/MIC) occur chiefly as a range of concentration, and these possibly explain the increased lethal activity of higher

concentration of these antibacterial extracts above the minimal inhibitory concentration (MIC).

A. sativum extracts were found generally to be bactericidal in nature, likely through its zeolite antibacterial and cellular defense activities (Walter, 2011; Yoshiriro and Hajime, 2012). The antibacterial effects complemented with nutritional properties of A. sativum are achieved via zeolite catalyst and cat ion exchange (James *et al*., 2009; Walter, 2011; Yoshiriro and Hajime, 2012). It also has porous molecular sieve cage to trap and capture particles as well as scavenge for free radicals, carcinogens, viral and bacterial particles and toss them out of the blood stream; and then causes blood pH value to be unaltered. The examples of *A. sativum* zeolite antibacterials are allicin, 4,4-bis(dimethylamino)-benzhydrol, phytoallexin, phytochelatin, ferrous-bis-glycinate, scordinin, allylmethylthiosulfinate, garlicine, dimethylsulfoxide, francolite, sodium-iron-chlorophyllin, bentonite, and glycinylcysteinylglutamic acid (glutathione disulfide, GSSH) (Walter, 2011; Yoshiriro and Hajime, 2012). They act as antioxidants handling environmental toxins and detoxifies trapped bacterial oxidants via chelation are said to be natural detoxicant (Walter, 2011; Appendix I). *A. sativum*'s effectiveness as broad spectrum antibacterial is mostly attributed to its zeolite phenomenon. Its chelating activity of nanopharmacologic heavy metals (Ag, Fe, Zn, Se, Mn), bactericidal effects and also stimulates immune system against challenging BM pathogens (Table 4.3; Appendix I). *A. sativum* extracts may reduce medical emergencies and mitigates sudden death in BM condition. Its zeolites act as natural antibiotic and natural cellular defense against diseases (James *et al*., 2009; Walter, 2011; Yoshiriro and Hajime, 2012). The antihemorrhagic and *in vitro* antibacterial activity of *A. sativum* coupled with its dietary supplements may provide a rational strategy for chemotherapy in bacterial meningitis.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

A. sativum extracts had been found to contain alkaloids, flavonoids, saponins, oil, carbohydrates, cardiac glycosides and terpenoids. It also has been found to possess potent vitamin compounds as nutritive agent such as protein, β-carotene, α-tecopherol, ascorbic acid and biotin. It exhibited potent antihemorrhgic and antibacterial activities which fight microbes due to its phytoconstituents, antioxidant properties, dietary medicines and coupled with zeolite herbominerals present. However, *A. sativum* extract can interact with the entire systems. It plays a vital role in human health, human nutrition as integrative medicine against bacterial meningitis pathogens. The free radical formation that occurs in the biological system, as meningeal pathogens invaded the blood stream or bacteria lyses components cause vital organs destruction. This triggered inflammatory response which led to dysfunctions of multiple organs and sudden death; this can be easily detoxified toxins and rejuvenated. Therefore, this study supports the traditional use of *A. sativum* as a phytotherapy in bacterial meningitis by locals is scientifically justified.

6.2 Conclusion

This study has succeeded in providing valuable scientific evidence of good nutritional profiles, potent antihaemorrhagic effect and antibacterial activity as a natural antibiotic and biodefensive agent. This experimental investigation has provided the scientific validation for the ethnomedical use of *A. sativum* as a remedy to treat bacterial meningitis locally.

6.3 Recommendations

- The findings of this study have shown the need for further in-depth investigations to establish the economic viability of exploiting *Allium sativum* to address health problems such as nocosomials.
- There is need to further explore and utilize full benefits of this miraculous medicinal plant. Research project should be made for commercial production of *A. sativum* fortified food product and nutraceuticals.
- Toxicological profile test for safety is required with further purification and characterization of active principles of the extract for economic purposes.
- The various principles in the extracts especially the JEAS and its natural zeolites may be isolated and their specific activities as well as their mechanism of action determined.
- *Allium sativum* extract could be therefore used to develop novel antibacterial therapy with nutritional formulations, in the light of emergence and re-emergence of devastating bacterial meningitis in Sub-Saharan Africa including Nigeria.

REFERENCES

Animal Use and Care Policy (2010). Research Policy Ahmadu Bello University, Zaria Pp 24-30 (http://www.abu.org/researchpolicy)

Andrew, J. P. and Martin, C. J. (2001). Meningococcal Disease, *Medical Book, Methods and Protocols*. Humana Press: Pg721.

Bill and Melinda Gates foundation (2011). Aimed reducing deaths due to vaccine-preventable disease. New trivalent (ACW135) meningitis vaccine developed by GlaxoSmithKline. http:// www.msfaccess.org/meningitis-vacccine.

Bostros, S., El-Lakkany, N., Seif, El-Din S. H., Sabra, A. N., Ibrahim, M. (2005). Effects of garlic on albino mice experimentally infected and therapeutic values of garlic treatment against schistosoma mansoni. *Journal of Tropical of Biomedicine*, 26(1): 40-50.

Calderon, C. B.and Sabundayo, B. P. (2007). Antimicrobial Classifications: *Drugs for Bugs. Antimicrobial Susceptibility Testing Protocols.* CRC Press, Taylor &Frances Group.

Clemetson, K. J. (2012). Platelets and Primary Haemostasis. *Thrombosis Research* 129(3): 220-224.

Corey, S. J., Burkhardt, A. L., Bolen, J. B. and Geahlen, R. L. (2011). Research Laboratory, Clotting time (Coagulation). *Journal of the American Medical Association; JAMA* 11(2) 153-160.

Cuevas, L. E., Jeane, I., Moleswort, A., Hart, C. A., Savony, E. C., and Cannor, S. J. (2007). Risk mapping and early warning systems for control of meningitis in Africa. *Vaccine*s, 25:12-7.

Cheesebrough, M. (2005). *District Laboratory Practice in Tropical Countries*. Part 2, Pg 62-71and 116-129.

Chan, G., Hardej, D., Santoro, M., Lau-Cam, C., Billack, B. J. (2007). Carboxyl-Ebselen- Based Layer-by- Layer file as Potential Antithrombolic and

Antimicrobial coating. *Journal of Biochemcal Molecular Toxicology*. 21(5): 252-64.

Chang, S., Sievert, D. M., Hageman, J. C., Boulton, M. L., Tenover, F. C., Downes, F. P., Shah, S., Rudrik, J. T., Pupp, G. R., Brown, W. J., Cardo, D. and Fridkin, S. K. (2003). Cimolai. MRSA and the environment: implications for comprehensive control measures. *European Journal of Clinical Microbiology and Infectious Diseases.* 27(7):481-493

Chen, D. Z., Patel, D. V., Hactbarth, C. J. (2000). Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. *European Journal of Biochemistry*. 39:3853-3874.

Daniel, B. L., Paul, H. D. and Patrick G. J., (2003). 5-Fluorouracil mechanism of action and clinical strategies. *Nature Reviews Cancer*, 3:330-338.

Davey, P. G. (2000). Antimicrobial chemotherapy. In Ledingham J. G. G., Warrell,.D.A. *Concise Oxford Textbook of Medicine*. Oxford: Oxford University Press. PP.1475.

Derrida, M. (2003). Garlic extracts Powder (50:1) (100:1) Allicia (*Allium sativum*) on Enteric Bacteria (Enterotoxin-induced Diarrhoea). *New York Science Journal*, 163(4):206-231.

Durak, I., Ozturk, H. S., Olcay, E. G. (2002). Effects of garlic extract supplementation on blood lipid, antioxidant parameters and atherosclerotic plaque formation process in cholesterol–fed rabbits' *Journal of Herbal Pharmacotherapy* 2(2): 19- 32.

Durak, I., Erdinc, D., Hanefi, O., Hasan, S., Mustafa, K., Bilal A. and Aslihan, A. (2004). Effects of garlic extract consumption on blood lipid and oxidant/oxidation. *The journal of Nutritional Biochemistry*, Volume 15, issue 6, pages 373-377.

Donnet, A. (2007), Hemorrhagic septicaemia bacteria, (*Neurology-Neurosurgery sychiatry* V. 78(12):54-59.

Dossett, J. H. (2001). The early management of suspected bacterial meningitis and treatment; investigation, treatment and prevention of acute bacterial meningitis and nosocomial septicaemia in neonates. *Linking* 291:159-170.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutical and prophylactic properties. Part1. *Tree for life Journal* Volume 1:5.

Fattouch, S., Imen, B., Belguitt, H. (2007). Preparation of aqueous garlic extract (AGE), Morphological and Biochemical changes of *Salmonella hadar* and fungi.*International Journal of Morphology* 44(62):231-38.

Ginsberg, L. (2004). Difficult and Recurrent Meningitis. *Journal of Neurology, Neurosorgery and Psychiatry* 75 Suppl 1: 116-21.

Goldberg, J. B. and Pier, G. B. (2000). The role pyocyanin in Pseudomonas aeroginosa infection. *Trends Microbiology*.8:514-520.

Greenwood, B. (2006). Pneumococcal meningitis epidemics in Africa. *Clin Infect Dis*, 43 (6):701-703.

Greiner, R. and Konielzny, U, (2006). Inhibition of Helicobacter pylori by garlic extract (*Allium sativum*). *The African Journal of Microbiology Research* (AJMR), 7 (46): 1.

Hackam, D. G., Wooward, M., Newby, L. K., Bhatt, D. L., Shao, M., Snuth, E. E., Donner, A., Mamdani, M, and Donketis, J.D, (2011), European publication, Pathogenesis and Pathophysiology of Hemorrhage, Bacterial Endotoxin inVital Organs and Cerebral Hemorrhagic Stroke. *European Journal of NeuroScience*124 (20):2233-42.

Hugo, A. D. and Russell, W. B. (2007): Clinical Resistance of the ESKAPE Pathogens, Bacterial resistance. As antibiotic development declines and resistance rises. Journal of Pharmaceutical Microbiology, Vol. 20 (3): 440-58.

IRIN Africa News (2009): *African Meningitis belt*, *Wikipedia.*

Jeeri, R. R and Thiambiano, S. R. (2008). Infections a Menigocoques Maladies Infectieuseset Africa. *West Africa Medical Bulletin.*pg123-134.

James, L. F., Stewart, A. L. and Erik, J. D. (2009). Effective treatment of chronic illness resulting from the long-time build up of heavy metals in the body, such as chelation therapy. Uses of naturally occurring zeolite to remove toxic substances. *Journal of Nutrition and dietary Supplements*, volume 1:11-18.

Joel, L. (2010). Vaccine Linking to Autism and Vaccine Associated Paralysis: *British Medical Toxicology, www.vaccineresistancemovement.org*

Johnson, A. (2000): Blood. *Journal of the American Society of Hematology*, Volume 83 (1):113-124.

Kingston, W. (2008). Irish contributions to the origins of antibiotics. Irish Journal of Medical Science 177(2):87-92.

Larguita, P. R. and Gemma, A. R. (2008). Phytochemical Screening of wellawel (Chromolaena odorata) leaves, its antimicrobial and coagulative properties. *JPAIR Multidisciplinary Journal*, Volume 1, No 1.

Lindblad, W. J. (2008). Considerations for Determining If a Natural Product Is an Effective Wound-Healing Agent. *International journal of Lower Extremely Wounds* 792): 75-81.

Lovera, D. and Arbo, A. (2005): Risk factors for mortality in Paraguay children with pneumococcal meningitis. *Tropical Medicine International Health*, 10(12):1235 – 41.

Madigan, M. and Martinko, J., (2005). Brock Biology of Microorganisms, 11th edition Prentice Hall, Pp 30-34.

Mark, A. S., Steven, H. W. and Craig, L. F. (2005): The *Laboratory rat - blood clotting time* pg 728.

Michael, S., Uwe, K., Barnett N. and Hans-Walter P. (2002). Pathophysiology of Bacterial Meningitis: *Journal of Infect Dis* Vol. 186 (2):5223-5233.

Molesworth, A. M., Cuevas, L. E., Connor, S. J., Morse, A. P. and Thomson, M. C. Environmental Risk and Meningitis Epidemics in Africa. *Emergence Infectious Disease* 2003; 9: 128-93.

Naturopathy, (2010). Chinese Medical Herbology and Pharmacology. *Chinese Complementary and Alternative Medicine* 4(3):33-240.

Nester, E. W; Aderson, D. G; Robert, C. E; Persal N. N.; Nester, N. N; and Hurley, D. (2004). Microbiology: *A human perspective*. 4 th edition McGraw Hill Coyp.Inc. PP 605-606.

Ncube, N.,S, Afolayan, A. J and Okoh, A. J. (2008). Assessment Techniques of Antimicrobial propertiesof Natural Compounds of Plant Origin Current Method and Future Trends *African Journal of Biotechnology* 7:1997-1806.

Nikaido, H. and Pagès, J. M. (2012). Broad-Specificity Efflux Pumps and Their Role in Multidrug Resistance of Gram-Negative Bacteria. *FEMS Microbiology Reviews*, 36: 340–363.

O'Brien, K. L., Watt, J. P., Mulholland, K., Birmingham, M., Henkle, E., Lee, E., McCall, N., Moisi, J., Tian, S., Mangtani, P. (2009). Paediatric Bacterial Meningitis; *Lancet, Journal of paediatric medicine* 11(11):374-398.

Oduye, T. (2011): National Health Holistic Consultancy: Garlic Constituents and its Organosulfur are Good Interactive Herbs for Mitigating Diseases, Amelias Organics Limited; The Nation, Thursday, April 28, pg 25.

Okoko, B. J., Idoko, O. T., Adegbola, R. A. (2009). Prospects and Challenges with Introduction of a Monovalent Meningococcal Conjugate Vaccine in Africa. *Medical Research Council Laboratories.* 27 (14): 2023-9.

Onipede, A. O., Onayade, A. A., Elusanya, J. B. E, Obiajunwa, P. O, Ogundare, E. O. O, Olaniran, O. O (2009). Invasive Bacterial Isolates from Children with Severe Infections in a Nigerian Hospital *Journal of Infectious Diseases*; **3**(6):429-436.

Overington, J. P., Al-Lazikan, B., Hopkin, A. L. (2006). How many drug targets are there? *Nat Rev Drug Discovery*. 5(12): 993-6 – Pubmed.

Pekthong, D. (2008). Garlic *(Allium Sativum)* in the treatment of arteriosclerosis and other vascular disease. *Journal of ethnopharmacology* 115: 432-440.

Peltola, H. (2000). World *Haemophilus influnzae* type b Disease at the Beginning of the 21stCentury: Global Analysis of the Disease Burden 25years after the use of the Polysaccharide Vaccine and a Decade after the Advent of Conjugates. *Clinical Microbiology Reviews* 13(2),302-17.

Pilkhwal, S., Sharma, S., Kuhad, A., Mukesh, L. S. (2010). The direct effects of bacterial endotoxin and circulation after killing bacteria. *International Journal of Phytomedicine*; 2(2010):47-5148.

Prescott, J. F., Baggot, J. D.; Walker, R. D. (2007). Assessment of the Antibacterial therapy in children on Compound Beta-lactams and Cephalosporin. *Pediatr Infect Dis Journal*; 26:377-81.

Rabih O.; Darouiche, M. O.; *et al*., (2012). Chlorhexidine-Alcohol versus Povidone-Iodine for Surgical-Site Antisepsis. *New England Journal of Medicine* 362(1):18-26.

Riodan, A. (2001). Current Opinion in Neurology, Neuroinflammation and Hemorrhagic Sepsis of Brain. *American Journal of Neurology*, 12: 301-304.

Rojas, J. J, Ochoa, V. J., Ocampo, S. A., and Munoz, J. F. (2006). Screening for Antimicrobial Activity of Ten Medicinal Plant Used in Colombia Folkloric Medicine: A Possible Alternative in Treatment of Non-nosocomial Infection. BMC Complement, *Alternative Medicine*, 6:2-9.

Rukangira, E. (2001). *Medicinal Plants and Traditional Medicinal in Africa*. 4th Edition, John Wiley Sons Ltd, Chichester pg 46-105.

Sachin, V. T. (2014). Ethanolic extract of *A. cepa* reported shorten the whole blood clotting time, plasma. *Journal of Medicinal Plant Research*; 8(3): 167-177.

Saez-Llorens, X. and McCracken, G. H., (2003). Bacterial Meningitis in Children. *Lancet American Journal of Infectious Diseases*, 361 (9375): 2139-48.

Schromm, A. B, Nagarakanti, R., Oldgren, J., Gunther, O., Mohan, H. (2000). Blood hemorrhage and bacterial endotoxin*, American Journal of NeuroScience* 66, 279:433-446.

Segal S, Pollard A. J (2004). Vaccines Against Bacterial Meningitis. *British Medical Bulletin* 72:65-81.

Sen Saikat, De Biplab, Devanna N., Chakraborty Raja (2012). vitro antihelmintic (antitrematodal) activity in ruminant from plant derived compounds

antioxidant, cysteine proteases and diallyl disulphide. *Pubmed*, www.science.gov/ garlic/vitro.

Sharma C, Aneja K. R. (2012). Agar well diffusion method, Antimicrobial potential of *Terminalia chebula* Retz fruit extracts, *World Journal of Otorhinolaryncology*, 78:42- 9.

Shyh-ming T. and Mei-chin Y. (2001). In Vitro Activity of Garlic Oil and four DiallylSulphides Against Antibiotic-Resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae Chinese Journal of Antimicrobial Chemotherapy* 47(5): 665-670.

Sindhu, S., Amuchou, S. S. and Kamala, S. (2011). Choice and duration of antimicrobial therapy for neonatal sepeis and meningitis. *International Journal of Paediatrics,* Volume 2011(2011):9.

Sultan, B., Labadi, K, Guegan J-F. and Janicot S. (2005). Climate Drives the meningitis epidemic Onset inWest Africa. *West African Journal of Infectious Disease*; 2: 43-9.

Sofowora, E. A. (2008). Medicinal Plants and Traditional Medicine in Africa. (3rd edition), Spectrum Books Ltd, Ibadan, Nigeria. Pg 199-205.

Surbhi, L., Chritine, L. T. and Randall, S. E. (2011). An introduction to the symposium on antimicrobial therapy. *Journal of Clinical Microbiology Review*. Vol.82(2): 156-167.

Stephens, D. S., Greenwood, B. and Brandtzaeg, P. (2007). Epidemic Meningitis, Meningococcal and *Neisseria meningitidis*. *Lancet British Journal of Infectious Disease*; 369: 2196-2210.

Stites, D. E. P., Stobo, J. D., Wells, J. V. (1987). Serum proteins and nutritional status of free- living. *Basic and clinical Immunology*, 6th ed., Los Altos, Calif; Lange medical publications. Appleton and Lange 1987; Pp241-84.

Tally, E. P., and DeBruin, M. F. (2000). Development of daptomycin for Gram-postive infections. *Journalof chemotherapy*.46 (4): 523 – 526.

Tebruegge, M. and Curtis, N. (2008). Epidemiology, Etiology, Pathogenesis and Diagnosis of Recurrent Bacterial Meningitis. *Clinical Microbiology Reviews* 21(3): 519-37.

Thomson, M. C., Molesworth, A. M., Djingarey, M. H., Yameogo, K. R., Belanger, F. and Cuevas, L. E. (2006). Potential of environmental models to predict Meningitis Epidemics in *African Tropical Medicine International Health*; 11: 781-8.

Trease, G. E. and Evans, W. C, (2002). *Textbook of Pharmacognosy* 15th edition, Elsevier Publication Ltd, pg 6-9.

Tunkel A. R., Hartman, B. J., Kaplan, S. L., (2004). "Practice Guidelines for the Management of Bacterial Meningitis". *Clinical infectious Disease* 39 (9): 1267-84.

United Nations (2012). Malnutrition Linked to Bacterial Meningitis Scourge in Africa, http;//*www.unitednation-malnutritionlinkmeningitis*, Health Experts.

USDA (2010). Garlic Database, *Wikipedia*.

Van, S. (2010). Proximate and Nutritional Composition Analysis of Plant Extract Using Analytical Instruments. *British Journal of Nutrition* 80:1-193.

Van de Beek, D., de Gans, J., Tunkel, A. R. and WiJdicks, E. F. (2006). Community Acquired Bacterial Meningitis in Adults. *The New England Oxford Journal of Medicine* 354(1): 44-53.

Vaccine Resistance Movement (VRM) studies (Joel, 2010). Phytotherapies are future remedies: *http//:www.vaccineresistancemovement.study.com.*

von Nussbaum, F., Brands, M., Hinzen, B.; Weigand, S.; Habich, D. (2006). 'Medicinal Chemistry of Antibacterial natural Products. Angew. Chem Int. Ed.45(31):5072-5129.

Walter, S. (2011). Chelation Therapy and Natural Detox: *Garlic Zeolite Review*.

Walker R. and Whittlesea C. (2008). *Clinical Pharmacy and Therapeutics*, 4th edition pg 531-33, Churchill.

World Health Organization, (2007). WHO-CDS-EPR, Malnutrition Links Bacterial Meningitis, *Wikpedia.*

World Health Organization-Biological Standardization, (2011). WHO Expert Committee on Biological Standardization-Bacterial Suspension, *Wikipedia.*

World Health Organization, (2009). Bacterial Meningitis (BM) in Sub-Saharan Africa, *Wikipedia*.

World Health Organization (2011). Media centre -Meningococcal meningitis, Fact Sheet *www.whofactsheet/meningitis/mediacentre*.

World Health Organization (2010). Traditional Medicine*, Wikipedia.*

Yoshiriro, I. and Hajime, H. (2012). Effect of natural zeolites against pathogenic organisms spread in air. *Journal of Biomedicine and Biology* Volume 3 (1):114-117.

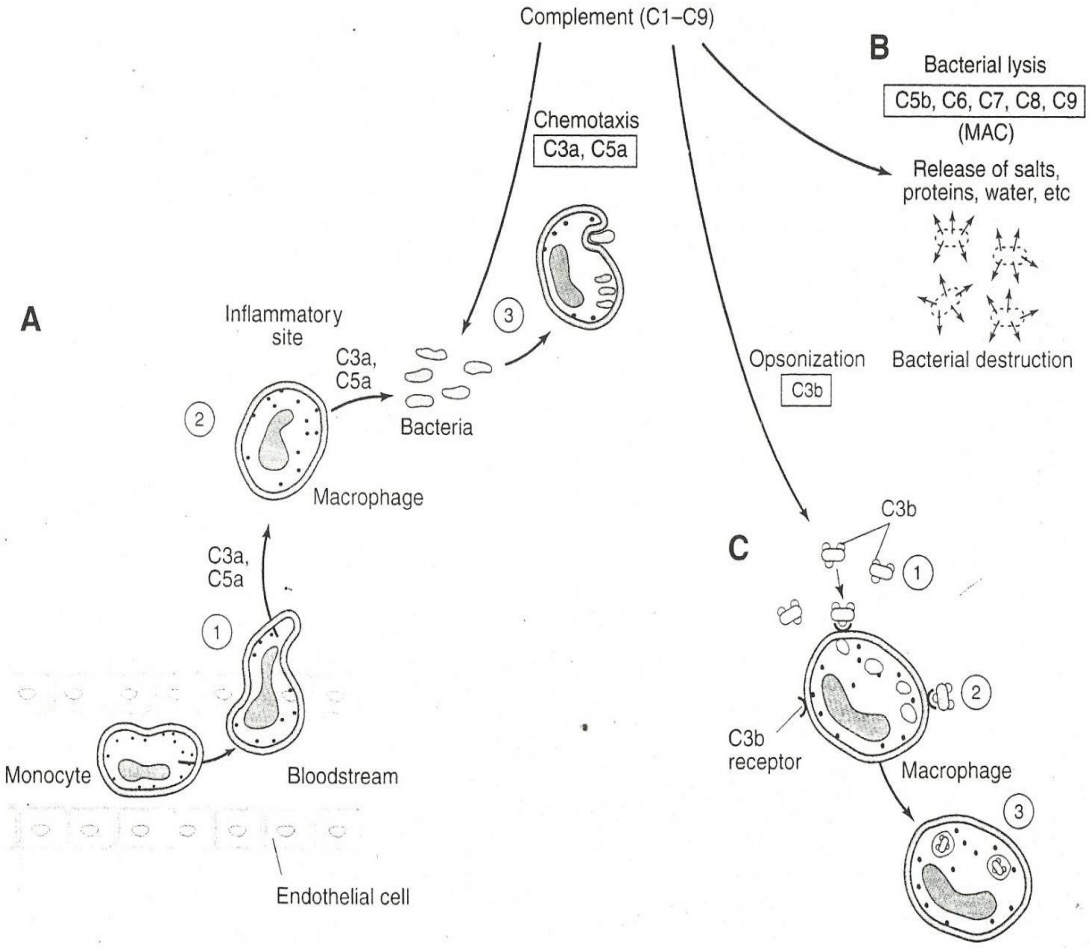
Yeo, J. S., Zheng, C. J., Lee, S., Kwak, J. H and Kim, W.G. (2006). Macrolactin N, a new peptide deformylase inhibitor produced by *Bacillus subtilis*. *Bioorganisation Medical Chemistry*. 16:4889 – 4892.

Yu, Q., Long, C., Lv, P., Shao, H., He, P., Shibata, N., Kajikawa, Y. and Sakata, I. (2012). Aminoacyl- tRNA synthetase inhibitors as potent antibacterials, *Current Medicinal Chemistry,* 19(21): 3550-63.

APPENDIX I

FIRST LINE OF IMMUNE DEFENSE SYSTEM: INNATE IMMUNE SYSTEM

Fighting toxins and ROS- Protein, vitamins A, H, C & E, Sulphur, Zinc, Selenium, Calcium, Ag, Mn and Fe (Zeolites)

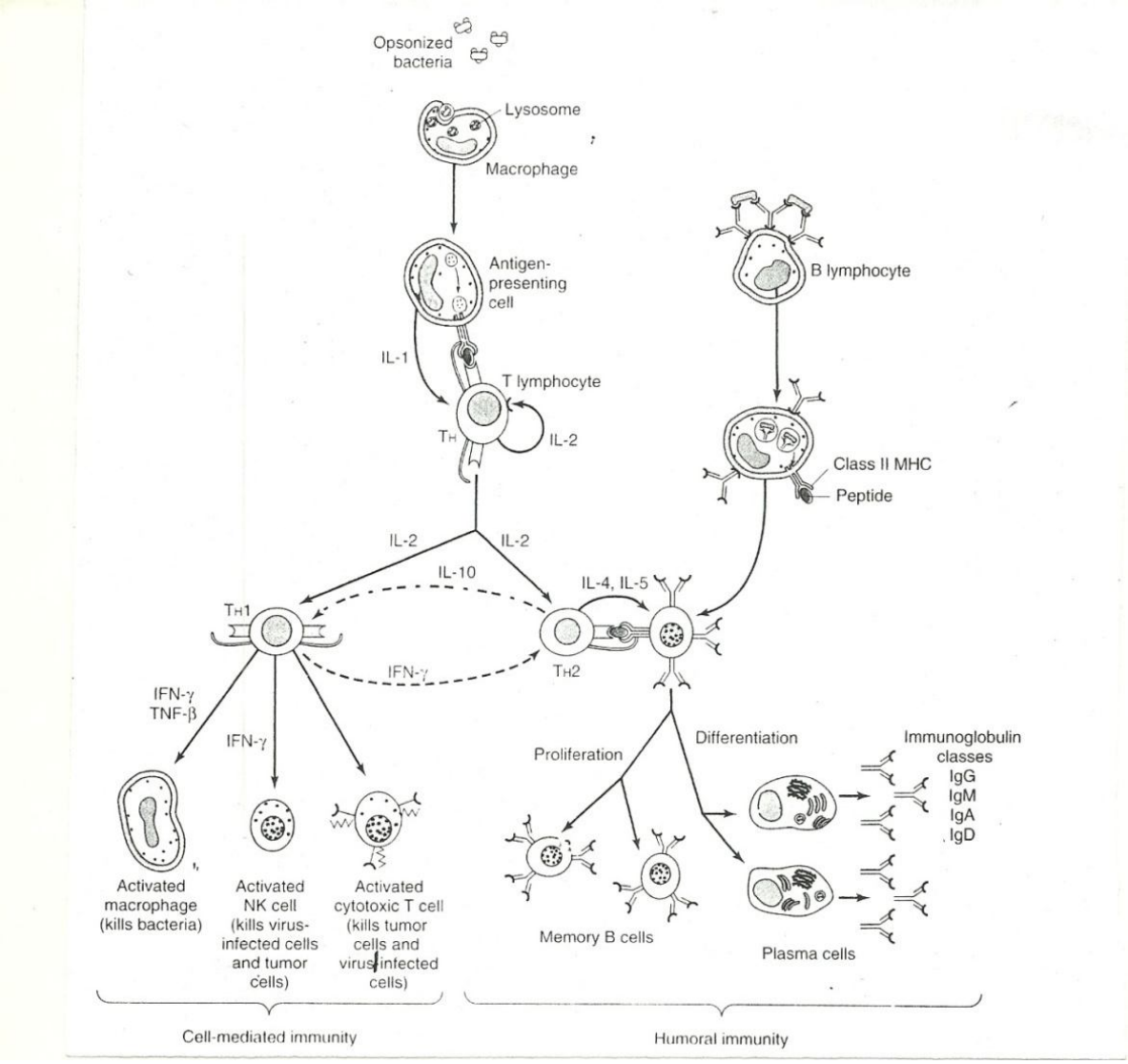


Source: Stites *et al*., 1987

Effects of nutrition powered complement in innate immunity. Complement is made up of nine proteins (C1-C9), which are split into fragments during activation. A: Complements (C3a, C5a) attract phagocytes (1) to inflammatory sites (2), where they ingest and degrade pathogens (3). B: Complement components C5b, C6, that C7, C8 and C9 associate to form a membrane attack complex (MAC) that lyses bacteria, causing their destruction. C: Complement component C3b is an opsonin that coats bacteria (1) and facilitates their ingestion (2) and digestion (3) by phagocytosis

APPENDIX II

SECOND LINE OF IMMUNE DEFENSE SYSTEM: ADAPTIVE IMMUNE SYSTEM



Source: Stites *et al*., 1987

This is followed if innate immune defense is breached, then adaptive immune defense will be provoked as a result of malnutrition. The bacterial endotoxins or neurotoxins will be attacked by cell-mediated and humoral immunes forming endotoxins-immunoglobin complexes that trigger inflammation and cell injuries in systemic and central nervous system of the patients.

APPENDIX III

ALLIUM SATIVUM L EXTRACT ANTIBACTERIAL SENSITIVITY USING AGAR WELL METHOD



Plate 2: *Haemophilus influenzae* zones of inhibition.

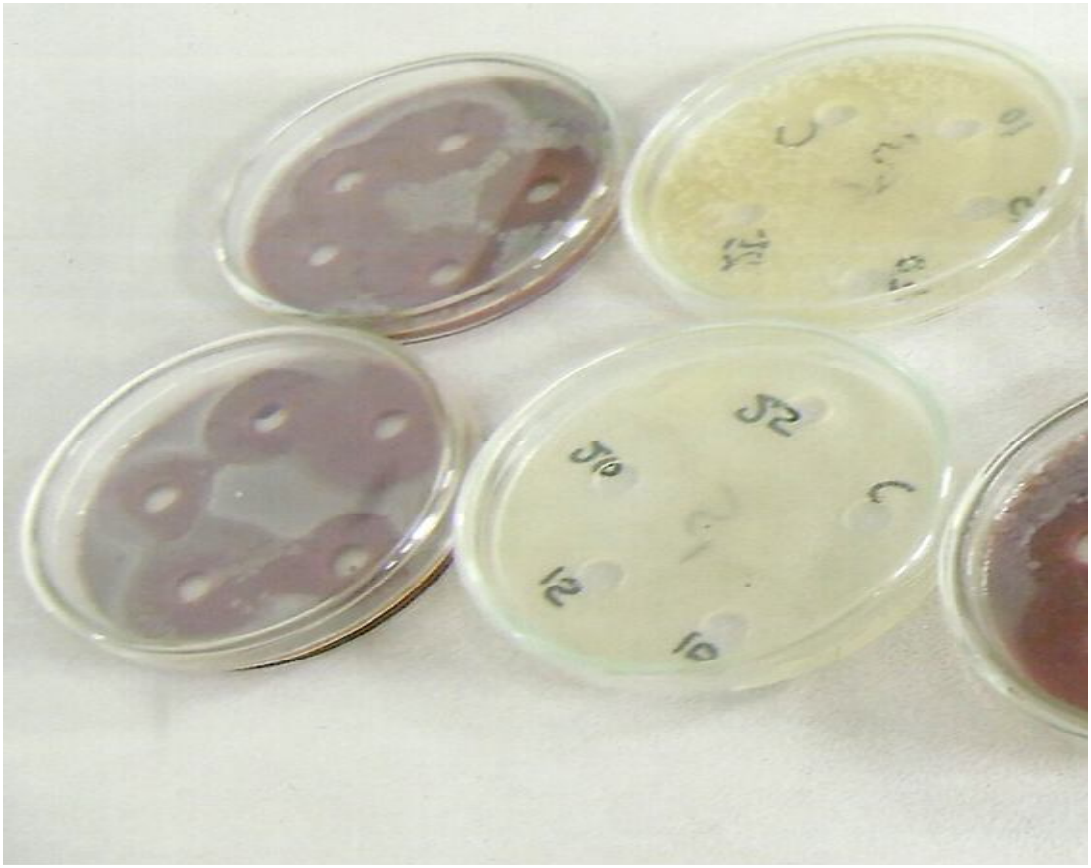


Plate 3: ***Streptococcus pneumoniae, Escherichia coli*** **and** ***Klebsiella pneumoniae*** **resistant.**

Zones of inhibition



Plate 4: *Neisseria meningitidis* zones of inhibition.