Evaluation of the antimicrobial activity of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* Hochst. ex. A. Rich. (Urticaceae)

By: Fentaye Kassa

A thesis submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology

Addis Ababa University

Addis Ababa, Ethiopia

December, 2016

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Fentaye Kassa, entitled "Evaluation of the antimicrobial activity of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* Hochst. ex. A. Rich. (Urticaceae)" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External examiner: ___________________________ Signature: _________ Date: ___________

Internal examiner: _____________________________ Signature: _________ Date: ___________

Principal advisor: Workineh Shibeshi (PhD) Signature: _________ Date: ___________

Principal advisor: Teshome Nedi (PhD) Signature: _________ Date: ___________

CO-advisor: Tadesse Eguale (PhD) Signature: _________ Date: ___________

CO-advisor: Aklilu Feleke (DVM, MSc) Signature: __________ Date: __________

____

Chair of Department

ABSTRACT

Evaluation of the antimicrobial activity of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* Hochst. ex. A. Rich. (Urticaceae)

Fentaye Kassa

Addis Ababa University, 2016

Most antimicrobials used in the reduction of common infectious diseases have now been rendered ineffective owning to development of resistance. To overcome this resistance problem, search of alternative antimicrobial agents from medicinal plants is highly encouraged. *Urtica simensis* Hochst. ex. A. Rich. is one of the medicinal plants traditionally used for the treatment of bacterial and fungal infections in Ethiopian folklore medicine. However, the antimicrobial activity of the leaves of this plant has not been scientifically validated. The aim of this study was therefore to investigate the antimicrobial activity of both 80% methanol extract and solvent fractions of the leaves of *Urtica simensis.* The 80% methanol extract was prepared by maceration and the fractions were obtained by successive fractionation of the 80% methanol extract with chloroform, ethyl acetate and n-butanol followed by distilled water. The antimicrobial activity were evaluated on eight bacterial and two fungal species using agar well diffusion method at concentrations of 200, 400 and 800 mg/ml in the presence of positive and negative control. Minimum inhibitory concentration was determined by broth micro-dilution method. Among the gram positive bacteria, the most susceptible specie was *Streptococcus pneumoniae* (MIC of 1.36 mg/ml). *Klebsiella pneumoniae* and *Aspergillus niger* were most susceptible species in gram negative bacteria and fungi with respective MIC values of 2.54 and 3.13 mg/ml. Least value of MIC, MBC and MFC were 1.36, 5.21 and 6.25 mg/ml respectively. In general, n-butanol fraction exhibited greater activity against gram positive bacteria while ethyl acetate fraction revealed greater activity against gram negative bacteria and fungi. Chloroform fraction showed least activity against most test organisms while aqueous fraction did not show activity against any of test organisms. This finding provides a scientific evidence for claimed traditional use of *Urtica simensis*.

Key words: antimicrobial activity, agar well diffusion, minimum inhibitory concentration, *Urtica simensis*

ACKNOWLEDGMENT

First of all, I am deeply indebted to the Almighty God and his mother Saint Virgin Marry, who gave me health and patience in completing this work.

Besides, I would like to express my deep appreciation and sincere gratitude to my advisors Dr. Teshome Nedi and Dr. Workineh Shibeshi for their valuable comments, follow up and unreserved guidance and support while I was working and writing my thesis.

Then, I would like to thank my co-advisors Dr Aklilu Feleke for his financial support, provision of laboratory media and standard discs; Dr. Tadesse Eguale for his support in laboratory work and constructive comments in the fulfillments of the requirements for the finalization of this thesis project.

My sincere gratitude also extends to Mr. Hailemeskel Meshesha and Ms. Fantu Assefa for their consistent help in the laboratory activities for the extraction of the study plant.

My heartfelt thank and appreciation also goes to Mr. Haile Alemayehu for his consistence and unreserved support in the laboratory procedures; Ms. Mahlet Arage for her kindness and help in the qualitative phytochemical screening of study plant.

In addition, my sincere and hearfelt thank also goes to Akililu Lemma Institute of Pathobiology (ALIPB) for their kind hospitality and permission to use premises and facilities of microbiology laboratory. My sincere gratitude also goes to Ethiopian Public Health Institute (EPHI) for their cooperation in providing sheep blood, bacterial and fungal standard species.

Finally, I would like to forward my gratitude to Addis Ababa University for funding this study and Wolaita Sodo University for sponsoring my postgraduate education programme.

Table of Contents

ABSTRACT iii

ACKNOWLEDGMENT iv

LIST OF ABBREVIATIONS/ACRONYMS vii

LIST OF TABLES viii

1. INTRODUCTION 1

1.1. Infectious diseases 1

1.1.1. Treatment of infectious diseases 3

1.1.2. Drug resistance 5

1.1.3. Mechanism of resistance 6

1.1.4. Control of resistance 7

1.2. Medicinal plants 7

1.2.1. Medicinal plants used in the management of infectious diseases 7

1.2.2. Bioactive compounds in medicinal plants 9

1.3. The experimental plant 11

1.4. Rationale for the study 14

2. OBJECTIVES 16

2.1. General objective 16

2.2. Specific objectives 16

3. MATERIALS AND METHODS 17

3.1. Materials 17

3.1.1. Chemicals and solvents 17

3.1.2. Media 17

3.1.3. Bacterial test organisms and standard antibacterial discs 17

3.1.4. Fungal test organisms and antifungal discs 18

3.2. Collection and authentication of plant materials 18
3.3. Preparation and extraction of plant materials 18
3.3.1. Extraction procedure for crude extract 18
3.3.2. Fractionation of the crude extract 19
3.4. Media preparation and inoculum standardization 20
3.5. Antimicrobial activity assay 21
3.5.1. Agar well diffusion method 21
3.5.2. Determination of minimum inhibitory concentration of extract and fractions 22
3.5.3. Determination of minimum bactericidal concentration of extract and fractions 24
3.5.4. Determination of minimum fungicidal concentration of extract and fractions 25
3.6. Preliminary phytochemical screening 25
3.9. Data analysis 27
4. RESULTS 28
4.1. Antibacterial activity 28
4.2. Antifungal activity 33
4.3. The minimum inhibitory concentration of extracts against bacterial species 35
4.4. Minimum bactericidal concentration of extracts 35
4.5. The minimum inhibitory/ fungicidal concentration of extract against fungi 37
4.6. Preliminary phytochemical screening 38
5. DISCUSSION 39
6. CONCLUSION 45
7. RECOMENDATIONS 46
8. REFERENCE 47

LIST OF ABBREVIATIONS/ACRONYMS

ATCC	American Type Cell Culture
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CLSI	Clinical Laboratory Standard Institute
DMSO	Dimethyl Sulfoxide
EPHI	Ethiopian Public Health Institute
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
SEM	Standard Error of the Mean
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

LIST OF TABLES

Table 1: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram positive bacteria………………………………………………..30

Table 2: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram negative *E. coli*, *S. Typhi* and *P. aeruginosa* bacteria…………...31

Table 3: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram negative *S. flexneri* and *K. pneumonia* bacteria………………….32

Table 4: Antifungal activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested fungi……………………………………………………………34

Table 5: MIC and MBC in (mg/ml) of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested bacterial species……………………………………………36

Table 6: MIC and MFC in (mg/ml) of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested fungal species………………………………………………37

Table 7: Preliminary phytochemical screening of the 80% methanol extract and solvent fractions of the leaves of *Urtica simensis*…………………………………………………………………38

1. INTRODUCTION

1.1. Infectious diseases

Infectious diseases which are caused by pathogenic microorganisms such as bacteria, viruses, fungi and protozoa have been the major health problems threatening human and animals throughout the world (WHO, 2012). Majority of the microbes on earth are benign and coexist peacefully with their host. These types of microbes are necessary for ecological stability; hence they are useful for human health and its survival. On the other hand, more than 1,400 pathogenic microorganisms cause disease in human beings (Mandell *et al*., 2005; Koenig and Schultz, 2014), and many of them cause serious infections particularly in tropical and subtropical countries of the world. Therefore, pathogenic microorganisms are absolutely required to infect human and to develop infectious diseases (Bellamy, 2004). These pathogens are entirely dependent upon their host machinery for resources and many of them are capable of surviving and rapidly expanding in a variety of host tissues. Thus, diseases can spread directly or indirectly from infected to susceptible person, which make infectious diseases as the leading cause of death worldwide (Nanuta and Mukeslr, 2012; WHO, 2012).

The term infectious disease applies when an interaction of microbial pathogens with the host organisms leading into host damage and alteration of physiology, which results in the development of clinical signs and symptoms of diseases. Infectious diseases caused by pathogenic species of bacteria include some of the most common infections in the past, present and probably in the future. The most common bacterial pathogens which cause infectious diseases include *Vibrio cholerae*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Aeromonas* spp., *Pseudomonas* spp., *Campylobacter* spp., *Klebsiella* spp., and *Staphylococcus aureus* etc. (Acharyya *et al*., 2009; Mandell *et al*., 2005). Pathogenic fungal organisms are also confronting human health and cause superficial or deep seated infections. In the last 30 years, the invasive type of fungal infections have been an increasing cause of morbidity and mortality mainly in immuno-compromised patients (Maertens and Marr, 2007; Mainous and Pomeroy, 2010).

A variety of microorganisms threat a human health. The ability of humans to control and prevent infectious diseases is continuously challenged due to the enormous diversity of microbial

pathogens combined with their ability to evolve and adapt to changing environment (CDC, 2011).

All human beings are at risk of infection caused by pathogenic microorganisms, disrespecting the socio-economic status of an individual, but the risk is not evenly distributed. In low income countries, the impact of infectious diseases is often devastating because infections decrease survival rate particularly among children. It also hinders the opportunity for economic growth and development. Poor people are more vulnerable to the impact of extreme weather events because of lack of food and lack of medical care once infected with microbial pathogens. The presence flimsy housing and inadequate early warning systems are also additional factors contributing to the vulnerability of poor people for infectious diseases (Alsan *et al*., 2011; WHO, 2013).

In general, humans are in a delicate balance with microbial cohabitants of the earth. Infectious disease occurs when the balance between human and microbes are troubled and tip the favor for microbial pathogens. In this regard, there are emerging and re-emerging infectious diseases. An emerging infectious disease occurs when new pathogenic vigor causes a new and previously undefined disease. Whereas, re-emerging infectious disease is caused by renewed pathogenic vigor that historically infected humans, but reappear at a new locations or drug-resistant forms after apparent control or elimination (Fauci and Morens, 2012; WHO, 2014). Moreover, most of infectious diseases caused by new or renewed pathogenic vigor are zoonotic in their origin that means; they emerged from an animal and crossed the species barrier to infect human beings. So far, infectious diseases which affected approximately 60% of all human beings and emerging infectious diseases which affect 75% the people over the past three decades were originated from animal sources (WHO, 2012).

Over the past few decades, infectious disease conditions which result from novel emerging pathogens have significantly increased. This rise in the incident of infectious diseases might be due to the alteration of various environmental, biological, socioeconomic and political factors. Severe acute respiratory syndrome (SARS), the 2009 pandemic influenza A H1N1, Ebola virus disease and Middle East respiratory syndrome are a few of many examples of emerging

infectious diseases events of international concern in the modern world (Fauci and Morens, 2012; Morens *et al*., 2004).

1.1.1. Treatment of infectious diseases

The discovery of antimicrobials is one of the most significant achievements of modern medicine and has considerably contributed in the reduction of common infectious diseases. Hence, these antimicrobial agents improved the health of the world and saved millions of lives specifically in the last half of the 20th century. A number of new antimicrobials have been developed and came into clinical use, and helped in the treatment of various types of human infections (Powers, 2004). Infectious diseases caused by bacterial and fungal microorganisms have been selectively treated using antibacterial and antifungal drugs respectively. But fungal infections are difficult to treat than bacterial infection because fungal organisms grow slowly and their cell components have more similar structure to mammalian cell (Anderson *et al*., 2012).

Antimicrobial agents use various mechanisms of action. Thus antimicrobials target a specific site of microbial pathogens. The well known mechanisms of action of antimicrobial agents include the interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of intermediary metabolic pathways and disruption of the cytoplasmic membrane (Tenover, 2006). Therefore, some antibacterial drugs like beta lactams (penicillins, cephalosporins and carbapenems) and glycopeptides (vancomycin and teicoplanin) inhibit bacterial growth by interference with cell wall synthesis. While other antibacterial drugs, inhibit protein synthesis either acting on 50S ribosomal subunit like macrolides (chloramphenicol, clindamycin and linezolid) or 30S ribosomal subunit like aminoglycosides and tetracyclines. Interference with nucleic acid synthesis either by inhibiting DNA synthesis (fluoroquinolones) and RNA synthesis (rifamycins) is also another mechanism of antibacterial inhibition. Sulfonamides and trimethoprim drugs of antibacterial inhibit intermediary metabolic pathways, while polymyxins and daptomycin drugs disrupt cytoplasmic membrane and increased the permeability of the cell (Liwa and Jaka, 2015).

The fungal cell wall structure contains mainly mannan, chitins and glucans in different proportions depending on the species of fungi. These structural components are unique to fungi and they are being investigated as targets for antifungal drugs since a long time. Therefore,

various classes of antifungal drugs act upon fungal cell structures with different mode of action. Among those, the azole class of antifungal inhibits lanosterol. Polyenes inhibit the binding to ergosterol. The DNA/RNA of fungi is inhibited by nucleoside analogues. Allylamines and thiocarbamates class of antifungals inhibit squalene epoxidase, whereas antibiotic like griseofulvin interact with β-tubulin of the cell structure and inhibit its proper functioning (Jampilek, 2016; Paramythiotou *et al*., 2014).

The glucan component of the cell wall has recently become the major target for therapeutic applications and development of antifungal drugs. Therefore, three classes of glucan inhibitors are in use in the current situation. The first classes of glucan inhibitors are the lipo-peptides which include the echinocandins, while the second classes of inhibitors are the glycollipids like papulacandins. The third class of glucan inhibitors is the acid terpenoids which includes the enfumafungin and ascosterosides (Aderiye and Oluwole, 2015).

Although the arrival of new antifungal and antibacterial agent has clearly advanced the management of fungal and bacterial infections, treatment failures still occur. Treatment failure is the major problem particularly in developing countries where numbers of peoples don't attend health facilities for their illnesses; instead they commonly use self medication of drugs existing at home that are prescribed for other ailments and drugs stored at unsafe place. Diagnostic difficulties of already established disease is also another factor contributing to treatment failure in the developing country since it leads to lack of appropriate therapy, over utilization of drugs and inappropriate administration strategies (Ararsa and Bekele, 2015; Maertens and Marr, 2007).

In order to overcome such treatment failure issue of antimicrobials, various strategies are suggested. Among those, proper selection of antimicrobials should be done based on the identity of pathogens at species or strains level and the susceptibility of the pathogens to the antimicrobials should be done *in vitro*. In addition, the potential toxicity of the drugs to the patient, the pharmacokinetics and pharmacodynamics of the drugs, the possibility of drug interaction, cost of drug and the convenience of administration site should be considered to avoid treatment failure issues (Hospenth and Rinaldi, 2015).

Therefore, safe and effective strategy of antimicrobial use involves selecting an appropriate and effective drug at the recommended dose and with the narrowest spectrum of antimicrobial

activity. Prescribing drugs only when it is needed, use antimicrobials with fewest adverse effects and lowest cost are also parts of safe and effective strategy of antimicrobial use. Moreover, the combination of antimicrobial drugs is sometimes recommended since combined drugs have a broad spectrum of activity, reduce adverse effects and improve drug penetration into selected infection sites, such as the central nervous system, lungs and peritoneum (Hessen and Kaye, 2004; Kumar *et al*., 2014).

1.1.2. Drug resistance

Drug resistance is accelerated by the overuse and misuse of antimicrobial agents in humans and animals throughout the word. It is a threat to the public health since it is associated with increased morbidity and mortality. Antimicrobial resistance has been a growing threat to the effective treatment of an ever increasing range of infectious diseases caused by bacteria, parasites, viruses and fungal microorganisms. Therefore, the overall problems of antimicrobial resistance include difficulty in the treatment of the patient, severe infections, complication, longer hospital stays and increased cost of the drugs (WHO, 2014).

Currently, most widely used antimicrobials are subjected to resistance. Development of resistance is not only restricted to the old and commonly used antimicrobials but also some newer agents also face the same challenge as soon as they are introduced in clinical uses (Taylor *et al*., 2014). Thus, if the problem of drug resistance is not reversed, 10 million people will be estimated to die annually due to the infections by drug resistance organisms in 2050 (Mendelson, 2015). The development of drug resistance and its estimated recent economic consequence is really disturbing. For example, in the United States alone, the yearly cost of antimicrobial resistance has been estimated 21 to 34 billion dollars which is accompanied by more than 8 million days of additional stay in hospital. In addition, the global effects of antimicrobial resistance currently cause falling of gross domestic product (GDP) of 0.4% to 1.6%. This reduction in the GDP is estimated to be worth many billion dollars (WHO, 2014).

The antibacterial resistance against widely used antibacterial drugs is also the major problem encountering the globe. For example, in Ethiopia the percentage of resistance development of different bacteria varies: the percentage *Escherichia coli* resistance to third generation cephalosporins of ceftriaxone and ceftazidim is 70% and 53% respectively. On the other hand,

respective resistance of *Klebsiella pneumoniae* to ceftazidim and ceftriaxone is 14% and 20 %. The percentage of resistance in the methicillin resistant *Staphylococcus aureus* (MRSA) has 31.6% (Anderson *et al*., 2012; WHO, 2014).

In general, the resistance of microbes against antimicrobial agents can be grouped as intrinsic or extrinsic resistance. The intrinsic resistance is found naturally among certain microbes without prior exposure to antimicrobial drugs. Naturally, microorganisms do not possess target sites for the drugs hence, the drug does not affect the microbes or the microbes have low permeability to the agent. This can happen because of the differences in the chemical nature of the drug and the microbial membrane structures. Extrinsic resistance on the other hand develops in previously susceptible microbial species following drug exposure. This type of resistance is more common in patients with prolonged treatment course and improved survival of chronically immuno-compromised individuals with multiple antifungal drugs. Extrinsic resistance more commonly develop as a result of altered gene expression (Kanafani and Perfect, 2008; Sosa *et al*., 2010).

1.1.3. Mechanism of resistance

Microorganisms have evolved genetic and biochemical ways of resisting antimicrobial agents. Hence, these microbes use various ways of resistance mechanisms against the antimicrobials in order to develop resistance. Therefore, the first drug resistance mechanism against antimicrobials is physiological mechanism by which they modify or destruct the antibiotics by enzymes, alteration of the antibiotic target sites, and changes in antibiotic uptake or efflux. Thus, many microorganisms use one or more types of these physiological resistance mechanisms. For example, *S. aureus* produces the enzyme β-lactamase and cleaves β-lactam rings of the antibiotics, which results inactivation of β-lactam based antibiotics while penicillin resistant *S. pneumoniae* alter target site at which the antibiotic can access in to the cell. Moreover, other organism like *P. aeruginosa* loss the porin site where imipenem antibiotic enters into the cell and decrease its uptake (Mainous and Pomeroy, 2010; Perlin *et al*., 2015).

On the other hand, the second mechanism of resistance is genetic basis of resistance which is mediated by the acquired genes whose presence in a cell usually expresses resistance phenotype. This type of resistance mechanism can be developed through single point mutations, major deletions or rearrangements and expression of a latent resistance gene. These in turn alters a

variety of phenotypes in the microbes including alteration of target sites, enhanced efflux mechanisms and changes in outer membrane proteins, which limit the access of drugs to the cell (Conly, 2002; Rasheed and Tenover, 2003).

While, the last mechanism of antimicrobial resistance is the inducible one hence, resistance arises during treatment with a given antimicrobial agent. For example, treatment of influenza A with rimantadine regularly results in the rapid emergence of resistant virus in the affected patient. Moreover, certain cephalosporins will trigger expression of high concentrations of this enzyme (Mainous and Pomeroy, 2010).

1.1.4. Control of resistance

Controlling microbial resistance to antimicrobial agents requires a comprehensive approach and the essential components of the approach includes: reducing inappropriate prescribing of antimicrobials both for human and animal, appropriate dosing and treating with the combinations of existing agents. Moreover, treating with appropriate drug in cases where the etiological agent of infection is known are also an important measures to reduce development and spread of antimicrobial resistance (Ghannoum and Rice, 1999; Mehta *et al*., 2014).

The transmission of resistant organisms is reduced through the application of enhanced infection control strategy; protect the hygiene of the environment and identifying trends of resistance within the microbial population through surveillance study (Liwa and Jaka, 2015).

In general, drug resistance can be overcome through the discovery of novel antimicrobials from various sources. Therefore, much effort has to be done towards analyzing the compound of antifungal and antibacterial properties of what is called natural compounds or natural bioactive compounds isolated from plants, other microorganisms or marine organisms (Arif *et al*., 2009).

1.2. Medicinal plants

1.2.1. Medicinal plants used in the management of infectious diseases

The use of medicinal plants in the treatment of infectious diseases is an old age practice; and several natural products derived from plants are used for treatment of numerous human diseases for thousands of years. Medicinal plants are used in many parts of the world as healing for

variety of human ailments (Dyubeni and Buwa, 2012). The traditional use of plant for the treatment of infectious diseases remained the most affordable and easily accessible source of treatment particularly in the primary healthcare systems of developing countries (Maroyi, 2013).

Different plant extract/ fractions used for the treatment of different bacterial infection and proved effective in the *in vitro* antibacterial activity testing. Among such plants, *Acacia nilotica* and *Sida cordifolia* (Mahesh and Satish, 2008); *Harrisonia abyssinica* Oliv and *Terminalia kilimandscharica* Engl (Cyrus et al., 2008); *Tetradenia riparia* (Ndamane *et al*., 2013); *Delonix regia* and *Achyranthus aspera* (Dhanalakshmi and Manimegalai, 2013); *Punica granatum* and *Woodfordia fruticosa* (Bajracharya *et al*., 2008) and *Asparagus adscendens* (Thakur and Sharma, 2015) are some of the plant with in vitro antibacterial activity.

Similarly, *Acacia nilotica* and *Sida cordifolia* (Mahesh and Satish, 2008); *Curcuma zedoaria* and *Piper betle* (Kawsud et al., 2014); *Azadirachta indica* (Radhika and Michael, 2013) and *Anastatica hierochuntica* (Daoowd, 2013), are some of the plant species with antifungal activity.

Different plants and plant parts are used for treatment of various human ailments in general; and for the treatment of infectious diseases in particular. The use of these plants and plant parts for the treatment of specific diseases is widely practiced throughout the world. Currently, the interest and demand in the usage and application medicinal plants has been increasing since nobody could be willing to use medicine that does not offer healing from certain illness (Maregesi and Mwakalukwa, 2015).

In general, the acceptance and demand of drugs from plant sources has been increasing since there is a wide spread belief that green medicine and plant derived drugs is safe as a chemical compounds present in herbal products are a part of the physiological functions of living organisms. On top of that, herbal products have better patient tolerance, being relatively less expensive, have cultural acceptability and lesser side effects (Nair and Chanda, 2007; Prasad and Ramakrishnan, 2012).

On the other hand, the use of medicinal plants for the healing of various diseases may be associated with a range of troubles like scarcity of valuable medicinal plants, lack of standardization in methods of preparation and improper dosing of plant preparations. Moreover,

the use of medicinal plants for healing of diseases may also be negatively affected due to poor storage conditions of the plant product after its preparation. Overall, these factors affect the efficacy and effectiveness of healing using plants and its parts (Njume and Goduka, 2012).

Medicinal plants offered remedies to common ailments, which range from common cold to complex pathological disorders including those relating to the respiratory, circulatory and genito-urinary systems. Therefore, herbal medicine plays a critical role in the treatment of various infectious diseases and life threatening condition. Among those, diarrhea, dysentery, wound, fever, skin infections, oral candidiasis, cough, vaginal candidiasis, tuberculosis, malaria, meningitis, sexually transmitted diseases, thyphoenteritis, intestinal helminthiasis and urinary infections which are some of ailments to human beings and able to treat by using herbal medicine (Fodouop *et al*., 2015; Mabeku, 2011).

All parts of a plant can be utilized for medication purpose like roots, bark, woody stems, leaves, flowers, fruits, resin and seeds. For many species, different parts of the same plant are used to produce different remedies for various diseases (Van and Tap, 2008).

Plants and plant parts can be processed and can be taken in different forms. Hence it is prepared and used in the form of teas, syrup, essential oils, ointments, salves, rubs, capsules and tablets. The above mentioned preparation may contain a ground or powdered forms of a raw plant part or its dried extract and used for the intended purpose (Benezie and Galor, 2011).

1.2.2. Bioactive compounds in medicinal plants

Plant cells produce primary and secondary metabolites. Primary metabolites like carbohydrates, lipids, chlorophyll and proteins are involved directly in growth and metabolism. These metabolites are produced as a result of photosynthesis and involved in cell component synthesis. On the other hand, most natural products which derived from primary metabolites such as amino acids, carbohydrates and fatty acids are generally categorized as secondary metabolites or bioactive compounds. These metabolites are not involved in metabolic activity of cell synthesis and they regarded as products of biochemical synthesis or side tracks in the plant cells (Wadood *et al*., 2013).

Even though, these bioactive metabolites are not involved in metabolic activity of cell synthesis, they have important function in the living plants. Hence, they are used as free radical scavenger during photosynthesis, attract pollinators or seed dispersers, or inhibit competing plants and ward off herbivore animals or insect attacks. That is why most species of medicinal plants seem to be capable of producing various secondary metabolites (Amin *et al*., 2013).

The results of preliminary qualitative phytochemical analysis of different plant extract/fractions give rise to wide variety of bioactive metabolites. For example, *Digitalis purpurea* and *Berberis aristata* plants include metabolites like alkaloids, saponins, coumarins, glycosides, tannins, phenols, reducing sugars, steroids and triterpenoids. The *Achyranthes aspera* plants also had similar types of metabolites with preceding plant, except the absence of glycoside in *Achyranthes aspera* (Shrestha *et al*., 2015). The qualitative tests of *Samadera indica* showed the presence of carbohydrates, proteins, phenolics, alkaloids, saponins, flavonoids, tannins and resins (Deepa *et al*., 2015).

The presences of bioactive metabolites in human and animal body elicit pharmacological or toxicological effects. Due to this reason, the identification and isolation of bioactive metabolites of plants being essential in order to understand the nature of the secondary metabolites responsible for its medicinal property that will be therapeutically active and efficacious (Annapurna, 2015; Bernhoft, 2010).

The most important of these bioactive metabolites of plants are alkaloids, glycosides, tannins, flavonoids, proanthocyanidins, anthraquinones, terpenoids, phenylpropanoids, saponins, steroids and phenolic compounds (Bernhoft, 2010;Wadood *et al*., 2013) as well as resins and lignins (Deepa *et al*., 2015). Therefore, medicinal plants have known to treat variety of infectious conditions in human, due to the presence of the above mentioned secondary metabolites shown to have antimicrobial, antifungal and antioxidant properties. In fact, antimicrobials derived from medicinal plants represent a new source of stable and biologically active components that can established a scientific base for the use of plants in modern medicine (Belakhdar *et al*., 2015; Paul *et al*., 2012).

1.3. The experimental plant

Urtica simensis belongs to Kingdom- *Planate*; Phylum- *Magnoliophyta*; Class- *Magnoliopsida*; Order- *Urticales*; Family- *Urticaceae* and Genus- *Urtica.* The family *Urticaceae* is commonly known as nettle family comprises with the list of 48 genera and more than 2000 species of plants. Geographically, the species of these plants are mostly found in the tropical and subtropical regions of the world. While, 30-45 species of these plants are found within the cosmopolitan distribution of temperate regions. The genus *Urtica* comes from Latin word "Urere" which means "burn". Nettles are often easily recognizable for humans after having experienced for its sting (Mabberley, 2008; Sharma *et al*., 2015; Wu *et al*., 2015).

Several species of the genus *Urtica* (especially the *Urtica dioica* L.) are used medicinally to treat a variety of ailments. The *Urtica dioica* has been used for hundreds of years to treat rheumatism, arthritis, gout, eczema, anemia, urinary tract infections, kidney stones, hay fever and early stages of an enlarged prostate (called benign prostatic hyperplasia). The plant has antimicrobial activity against wide variety of bacteria and fungi organisms. Beyond its antimicrobial activity, the plant also has antioxidant, antiinflammatory, antiulcer and analgesic effect (Gulcin *et al*., 2004; Ramtin *et al*., 2014). According to Kavalali, (2003) there are approximately fifty active chemical compounds in the *Urtica dioica* plant including sterines, coumarins, simple phenols, triterpenic acids, lignans, hydroxyl fatty acids, and ceramides which might be responsible for the medicinal use of the plant.

Urtica simensis is one of the species of nettle which is endemic in Ethiopia. The plant grow in the highlands of Ethiopia especially in the North and South Gondar, North and South Wello, North Shewa, Wag Hamra, highland of Sidama zone in Southern region and Arsi zone of Oromia region throughout the year at 1500-3500 meter above see label. It is mostly found near houses (Dereje *et al*., 2016; Erenso and Maryo, 2014; Kavalali, 2003).

The plant commonly known as Nattle (English), Sama (Amharic), Dobii (Gurgubee) Gurgubbee/ Dobii (Oromifaa), Dobita (Kembatissa), Ameie (Tigrigna) (Alemayehu *et al*., 2015; Gebrezgabiher *et al*., 2013; Maryo *et al*., 2015; Tura *et al*., 2014).

Urtica simensis is dark green perennial wild species of plant traditionally used for food particularly during drought. It has a great potential and contribution to food security to meet nutritional demand of human. The leaves and young shoots of this indigenous plant cooked and eaten as vegetable because of having high nutritive contents, the plants grow throughout the year and available on demand in nearby area (Eskedar *et al*., 2013; Grublwn and Denton, 2004).

Urtica simensis is widely known for its unpleasant stinging hairs located under the stems and lower leaf surface. The whole plant is covered with stinging hairs. The plant is one meter tall, dioecious, erect and non-branched herbal nettles. The leaves are opposite simple, stipules fused and interpetiolar 0.5-1 cm long. The leaves bases are slightly cordate, the apex broadly acute and the margin of the leaves are also serrate. The flowers *Urtica simensis* are unisexual and regular, while the fruit is about 2 mm long (Gebrehiwot and Hundera, 2014; Grublwn and Denton, 2004).

Figure 1: photograph of *Urtica simensis* plant

As described in different ethnobotanical studies on traditional medicinal use of *Urtica simensis* plant in Ethiopia, different parts are used for the treatment of different illness and disorders. For the treatment of gonorrhea, root and leave part of *Urtica simensis* of plant is powdered in to smaller pieces, mixed in water and drunk the filtrate. Washing of the affected area of the body using root and leaves infusion once daily also practiced for the treatment of gonorrhea

(Alemayehu *et al*., 2015; Kefalew *et al*., 2015). On the other hand, the leave and young twigs of the plant is crushed and the resulting leaf juice is creamed with butter topically in the management of wound infections (Chekole *et al*., 2015; Enyew *et al*., 2014; Gebrezgabiher *et al*., 2013). The fresh leave of *Urtica simensis* is also used for the treatment of gastritis in two forms of preparation. Hence, the leaves is roasted; ground in to smaller pieces and the resulting juice was taken orally or the fresh leaves could be cooked and eaten by 'injera' (Chekole *et al*., 2015; Reta *et al*., 2015). In addition, the plant is reported to be used for the management of acute stomachache (drunk the sap orally) and body swelling (heat and put the leaf on the affected area topically) (Alemayehu *et al*., 2015), common cold (fresh root ground and taken orally) (Maryo *et al*., 2015), Rh-factor and heart failure (fresh leaf steam vapor allowed to enter nasally and fumigated whole body) (Enyew *et al*., 2014).

1.4. Rationale for the study

Bacterial and fungal infections are the most common causes of illness and sometimes cause a very serious problem in human. Several synthetic or synthetic derivative antimicrobials are in use to treat different diseases caused by human pathogenic microorganisms. It has been observed that the modern medicines are not much effective in the treatment of infectious diseases due to the alarming world wide spread of drug-resistant microorganisms (Abdullah *et al*., 2013). Moreover, administration of antimicrobials leads to the accumulation of metabolites in tissues and fluids causing toxicity and adverse side effects/ reactions, forced scientists to find new antimicrobial substances and search for new infection fighting strategies (Altinyay *et al*., 2015). Due to these reasons, plant derived compounds or phytomedicines having antibacterial and antifungal action are required to be evaluated and introduced in the treatment of infectious diseases (Shaik *et al*., 2014).

There are many plants in Ethiopia and throughout the world which are traditionally used for the treatment of diseases associated with bacterial and fungal caused infections, among which *Urtica simensi* is one of them. Therefore, this study attempted to validate the traditional use of this endemic medicinal plant and for further assurance of whether the crude or fractional constituent (s) is responsible for antibacterial and antifungal activity. Also, the study aimed to provides a clue about the qualitative phytochemical constituents of the plant so as to get insight into the nature of phytochemicals responsible for its action. The finding of this research could be used as

an input in searching of new antibacterial and antifungal agent that might solve problems associated with the conventional antimicrobial drugs.

2. OBJECTIVES

2.1. General objective

To evaluate the *in vitro* antimicrobial activity of 80% methanol extract and solvent fractions of *Urtica simensis* leaves on selected bacterial and fungal species.

2.2. Specific objectives

- ❖ To determine zone inhibition of 80% methanol extract and solvent fractions of *Urtica simensis* leaves on selected bacterial and fungal species using agar well diffusion method.
- ❖ To determine the minimum inhibitory concentration of 80% methanol extract and solvent fractions of *Urtica simensis* leaves on selected bacterial and fungal species using broth micro-dilution method.
- ❖ To determine the minimum bactericidal and minimum fungicidal concentrations of 80% methanol extract and solvent fractions of *Urtica simensis* leaves on selected bacterial and fungal species.
- ❖ To qualitatively determine the phytochemical constituents of 80% methanol extract and solvent fractions of *Urtica simensis* leaves.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and solvents

The chemicals used for the study were absolute methanol and n- butanol (Carlo Erba reagents, France), ethyl acetate (HiMedia Laboratories Pvt. Ltd., India), chloroform (Finkem Laboratory Reagent, India), 0.5 McFarland equivalence/standards (Remel, Lenexa Kansas 66215, USA), Dimethyl Sulfoxide (DMSO) (Uni-Chem, India) and resazurin sodium salt (Serva Feinbiochemica Heidelberg, New York). All the chemicals and solvents were of analytical grade.

3.1.2. Media

The bacteriological and fungal media used for this study were Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), brain heart infusion agar, blood agar base, mannitol salt agar and XLD medium (Oxoid Ltd, Basingstoke, Hampshire, England), brain heart infusion broth (Becton Dickinson and , Cockeysville), violet red bile glucose agar (Research-lab Fine Chem. Industries, India), Potato Dextrose Broth (PDB) (HiMedia Laboratories Pvt. Ltd. India), Potato Dextrose Agar (PDA) (Sisco Research Laboratories Pvt. Ltd. India) and sterile sheep blood (Ethiopian Public Health Institute (EPHI). All the media were used as the manufacturer guidelines.

3.1.3. Bacterial test organisms and standard antibacterial discs

The standard American Type Cell Culture (ATCC) bacterial species of *Streptococcus pyogenes* (ATCC 19615), *Streptococcus pneumoniae*) (ATCC 49619), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC) 25922) and *Salmonella typhi* (ATCC 13062) were obtained from Aklilu Lemma Institute of Pathobiology laboratory while, *Staphylococcus aureus* (ATTC 25923) and *Klebsiella pneumoniae* (700603) were collected from Ethiopian Public Health Institute (EPHI) microbiology laboratory.

The standard antibacterial discs used for the study were amoxicillin (30 µg/disc), ampicillin 10 µg/disc (Oxoid Ltd, Basingstoke, Hampshire, England) and ciprofloxacin 5 µg/disc (Becton, Dickinson and Company, USA).

3.1.4. Fungal test organisms and antifungal discs

Similar to bacterial test organism, the *Trichophyton mentagrophytes* (ATCC 18747) and *Aspergillus niger* (ATCC 10535) fungal test organisms were obtained from EPHI. The standard antifungal disc used in this particular study was nystatin 100 units/disc (Oxoid Ltd, Basingstoke, Hampshire, England).

3.2. Collection and authentication of plant materials

The leaves of *Urtica simensis* were collected in December 2015 around 4-kilo, Arada sub-city, Addis Ababa, Ethiopia. Botanical identification and authentication were done by Mr. Melaku Wondafrash at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University and a voucher specimen was kept there for future reference with voucher No. of 003/HR.

3.3. Preparation and extraction of plant materials

After collection, the leaves were initially washed using running tap water to remove dirt or dust and dried under shade in pharmacology laboratory within the school of pharmacy. The leaves were then chopped into small pieces manually and ground into coarse powder mechanically using a clean mortar and pestle. The powder sample was weighed and stored in air tight containers until extraction commenced.

3.3.1. Extraction procedure for crude extract

The crude 80% methanol extract of leaves of *Urtica simensis* was prepared by cold maceration. It was prepared by weighing 800 grams of coarsely powdered leaves using sensitive digital weighing balance (Mettler Toledo, Switzerland) and soaked in a clean flask containing 80% methanol in distilled water (200 g in 1600 ml) i.e. (1:8 (w/v)). It was then, kept for a period of three days accompanied by intermittent shaking using mini orbital shaker (Bibby Scientific Limited Stone Staffo Reshire, UK). The entire mixture was first filtered through a funnel plunged with muslin cloth two times and then the filtrate was passed through Whatman filter

paper (Number 1) (Maidstone, UK). After filtration the remaining residue or marc was re-macerated twice for a total of six days with a fresh solvent of 80% methanol. The marc was pressed, and the resulting solution after successive filtration was evaporated using a rotary evaporator (Buchii model R-200, Switzerland) set at 40^0C to remove methanol. Finally the concentrated aqueous solution filtrate was placed in deep freezer set at -20 ºC to solidify and dried in a lyophilizer (Operan, Korea vacuum limited, Korea). Evaporation of the plant extract gave a solid dark blue colored coarse powdered extract of 13.75% yield and kept in air tight container until used as it is or after solvent fractionation for the intended experiment.

3.3.2. Fractionation of the crude extract

Fractionation of crude extract was undertaken using solvents of different polarity like chloroform, ethyl acetate, n-butanol and water. The solvent fractionation was done by measuring 70 gram of crude (80% methanol) extract, suspended in 300 ml of distilled water, and then shaken to mix completely with solvent. About 300 ml of mixture (extract and solvent) was added in to funnel, and equal amount of chloroform was added to it. Then shaken gently to mix and allowed to settle for some times until it forms two layers (water at the top and chloroform at the bottom). The chloroform fraction was collected in a separate flask. More chloroform was added to take the almost the entire chloroform fraction and the procedure repeated twice as described above. Finally, separated chloroform portion was taken and evaporated using a rotary evaporator (Buchii model R-200, Switzerland), the filtrate was placed in an oven until dried, and left 31.75 % of dried powder of chloroform fraction. From the remaining filtrate within the separating funnel, equal amount of ethyl acetate (300ml) was added and mixed thoroughly. After settled for some time, the mixture was separated in two layers (ethyl acetate at the top and water at the bottom). Collect the ethyl acetate fraction in flask. To get a better yield fraction, ethyl acetate solvent was added twice and the procedures repeated. Then separated ethyl acetate portion was taken and evaporated using a rotary evaporator, the filtrate was placed in an oven until dried and left 21.80% of dried powder of ethyl acetate fraction. For the third solvent fraction, the upper separated layer of aqueous was taken into a separating funnel and 300 ml of n-butanol was added into it and followed by vigorous shaking as described for the preceding solvent fractionation. The upper layer was n-butanol, which was separated from aqueous portion and the procedure repeated twice. Then separated n-butanol portion was taken and evaporated using a rotary

evaporator and the filtrate was placed in an oven until dried. The total yield of n-butanol fraction was 12.5%. The remaining aqueous layer was collected and concentrated in a lyophilizer (Operan, Korea vacuum limited, Korea). The dried aqueous fraction yield was 34.3 %.

The re-constitution of both crude and solvent fraction of was conducted by using Dimethyl Sulfoxide (DMSO) so as crude and n-butanol were re-constituted using 20% DMSO while 40% DMSO for ethyl acetate and chloroform. But, the re-constitution of aqueous fraction was performed using sterile distilled water.

3.4. Media preparation and inoculum standardization

Both general purpose and selective media were prepared and used according to the manufacturers' guideline. Measured amount of media was added to flask containing known volume of distilled water, placed into hot plate stirrer until it boils. Most bacteriological and mycological media were placed in the autoclave and sterilized at 121 ºC for 15 minutes with the exception of violet red bile glucose agar and XLD medium where there was no need of sterilization after boiling. The medium was cooled to 45-50 ºC in water bath and 5% sterile sheep blood was added to blood agar base medium for fastidious *Streptococcus* species that require enriched media. Then, the media was poured into different size petri-dishes under aseptic condition inside Bio-safety Cabinet (Bioair instruments, Eurolone Company, Italy) and allowed some time to solidify. Similarly, broth media was prepared in test tubes for standardization with 0.5 McFarland equivalence and used for both agar well diffusion test and MIC determination.

Then, standard bacterial species were streaked and inoculated into prepared bacteriological agar media and incubated at 37 ºC overnight for all bacterial species. After overnight incubation, 3-5 well isolated colonies were streaked using inoculating wire from young colony growth and dipped in to test tube containing 4 ml of Mueller Hinton Broth (MHB), except of *Streptococcus* species as they were standardized in a test tube with 4 ml brain heart infusion broth (Buller *et al*., 2014; Nomura *et al*., 2006).

On the other hand, both fungal species were streaked into prepared PDA and incubated at 30 ºC for a period 48 hours. Then, few well isolated colonies were streaked into a test tube containing Potato Dextrose Broth (PDB) (Arevalo *et al*., 2003).

The bacterial and fungal turbidity standard was prepared according to Arevalo *et al*., (2003) and Balouiri *et al*., (2016) as follow: after the colonies were dipped into pre-labeled test tube, the test tubes were incubated 2-8 hours depending on the growth nature of the organism until visible turbidity was seen. Then, the organism suspension was adjusted to commercially available 0.5 McFarland standard which corresponds to 1×10^8 CFU/ml for bacterial and $1\text{-}5 \times 10^6$ for fungal species either by adding sterile normal saline (0.85% w/v) (dense suspension) or by adding more colony (clear suspension). In order to assess the visual comparison of 0.5 McFarland standard and tube suspension of the organism, the test and standard was compared with a white background with contrasting black line in the presence of adequate light. Then, the standardized suspension was used with 15 minutes of its preparation.

3.5. Antimicrobial activity assay

The effect of plant extracts on several bacterial and fungal species were assessed by agar well diffusion and micro-dilution methods. The agar well diffusion method was used in the measuring and determination of zone of inhibition of plant extract against test organisms, while micro-dilution method was used in the determination of minimum concentrations of the plant extracts needed to inhibit the growth of microbes.

3.5.1. Agar well diffusion method

The antimicrobial activities of plant extract were tested using agar well diffusion method (Sen and Batra, 2012). About 70 ml freshly prepared sterile MHA (bacteria) or PDA (fungus) media were poured into 150 mm diameter agar plate and allowed to cool at room temperature. Within 15 minutes of adjusting the turbidity of the inoculum suspension to 0.5 McFarland standard, a sterile cotton swab was dipped into adjusted microbial suspension, rotated gently and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was streaked to the entire surface of the MHA/PDA plate three times by rotating approximately 60° each time to ensure even distribution of the inoculum. Petri-plates were left for 3 to 5 minutes at room temperature (Dey *et al*., 2010). Then, an equal distance hole with a diameter of 6 mm was punched aseptically using sterile cork borer tip. A 100 µl volume of both 80% methanol extract and solvent fractions at concentration of (200, 400 and 800 mg/ml) were

introduced to fill the wells using micropipette after dissolved in DMSO (Kuta *et al*., 2015; Radwan *et al*., 2015).

The next step was the addition of positive and negative controls so as the positive antibacterial discs like amoxicillin (30 µg/disc) was used for *S. aureus* and *E. coli.* Ampicillin (10 µg/disc) was used as positive control for *Streptococcus* species. Ciprofloxacin (5 µg/disc) was placed as a positive control disc for the rest of bacterial species. The standard antibacterial discs were selected based on the susceptibility of bacterial species (CLSI, 2014). A 100 µl of DMSO was used as a negative control. After placement of the plant extracts and negative control into prepared wells and positive control into label area of the agar plate, the plates were placed undisturbed at room temperature for 2 hours (Sen and Batra, 2012). Then, plates were incubated at 37 ºC for 18-24 hours.

On the other hand, for both fungal species, nystatin (100 units/ disc) was used as a positive control. Similar to bacterial test organisms, 100 µl of DMSO was used as a negative control. Then, petri-plate was incubated 30 ºC for a period of 48 hours.

Then, complete zone of inhibition was measured in millimeter for extracts, fractions and disc of standards as judged by the naked eye using ruler. All tests were performed in triplicate for each bacterial and fungal species. The mean zone of inhibition and standard error of the mean (Mean± SEM) was calculated for the 80% methanol extract and solvent fractions as well as for standard antibacterial and antifungal discs.

3.5.2. Determination of minimum inhibitory concentration of extract and fractions

Minimum Inhibitory Concentration (MIC) is least concentration of the extracts that inhibit growth of microorganisms. MIC was determined by using broth micro-dilution method in 96 wells micro-titer plates (Costar, USA) as described previously by (Barsi and Fan, 2005; Singh *et al*., 2015), and done for those plant extracts which showed antibacterial and antifungal activity greater than or equal to7 mm zone of inhibition in agar well diffusion assay (Taye *et al*., 2011).

Sterile, 100 µl MHB for bacterial and equal amount PDB for fungal test species was added into each 96 wells of micro-titer plates using sterile automatic pipette (Medical diagnostic in vitro

device, Italy), so that each well hold 100 µl of growth medium (Singh *et al*., 2015). Momentarily, two fold serial dilutions of both 80% methanol extract and solvent fractions were performed after measuring 400 gram of powdered plant extract and adding 1ml of DMSO as solvent (concentration 400mg/ml). Then, highest concentration of the plant extract and fractions (100 µl) were added to the first well of the first column that already had 100 µl of growth medium. After proper mixing with broth media using automatic pipette, 100 µl of extracts and media mixture was transferred to the second well and in this way, the dilution procedure was continued for the subsequent wells until 10th column. From the 10th column, 100 µl of media and plant extract mixture was disposed so as each wells had 100 µl. This serial dilution of plant extracts from column number 1 (1st column) to column number 10 (10th column) was ranged from 200mg/ml to 0.39 mg/ml concentration in descending order. Hence, three similar concentrations were placed in three consecutive rows for one organism to determine the MIC in triplicates.

Similar to agar well diffusion method, the standardization of bacterial and fungal suspension were made in comparison with 0.5 McFarland standards. Then, within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standard, 2 ml of original suspension was added into 38 ml of MHB broth (1:20 dilution) so that the final concentration in each wells of bacterial suspension was 5 x 10^5 CFU/ml. On the other hand, 1 ml of fungal suspension was added to 99 ml of PDB (1:100 dilutions) to obtain the final concentration of 1-5 $\times$ 10^4 CFU/ml (Scorzoni *et al*., 2007).

Then, 20 µl of diluted bacterial and fungal suspension was added to each well (2 species per plate), the first three row for one organism. The fourth row was filled in all solution except the bacterial and fungal inoculum, which was replaced by 20 µl of MHB or PDB, was used as color contrast control for the first test organism.

While, row 5-7th were filled in 20 µl diluted organism suspension and the 8th row was filled in all solution except the bacterial and fungal inoculum, which was replaced by 20 µl of MHB or PDB, was used as color contrast control for the second test organism. The filling of the walls were done to 11th column for all test organisms. The wells of two column i.e. column 11th and 12th were used as growth and a sterility control respectively (Aly *et al*., 2012). In addition to the

broth, the growth control column was filled with 20 µl of test respective test organisms, whilst the sterility control column (12th column) was filled only with a 100 µl of appropriate growth medium.

Finally, 30 µl of resazurin sodium salt solution (Serva Feinbiochemica Heidelberg, New York) was added to each 96 wells of plate. The addition of resazurin reagent was done in strict precaution to prevent the contamination from one well to the other. Then, the plates were wrapped loosely with parafilm (Pechiney Plastic Packaging, Chicago) and placed in an incubator. Any color changes from purple to pink or colorless were recorded as positive and the lowest extract concentration that prevented this color change was taken as the MIC value. The test was performed in triplicate for each test organisms. A change in color of growth control wells to pink indicated the proper growth of the isolate and no change in color of sterility control column wells indicated absence of contaminants (Khalifa *et al*., 2013).

3.5.3. Determination of minimum bactericidal concentration of extract and fractions

Minimum Bactericidal Concentration (MBC) of both 80% methanol extract and solvent fractions of study plant against standard bacterial species was determined from wells of plates which showed no visible growth in MIC assay. Hence, the MBC of plant extracts were determined by taking a loop full of samples from wells having no visible growth in MIC assay and sub-cultured into antibiotic free agar medium (Chukwudi and Mohammed, 2013; Mathur, 2013). In this procedure, the loop was dipped in to each wells having a concentration of plant extract/fractions greater than or equal to MIC and inoculated to agar medium (MHA) for all bacteria except *Streptococcus* species. But, for *Streptococcus* species MHA enriched with 5% sterile sheep blood were used for sub-culturing.

Then, all test bacterial species were incubated at 37 ºC for 18-24 hours. The least concentration of the plant extract/fractions which inhibits the colony formation on solid agar medium was considered as MBC. The entire test was done in triplicate for each bacterial species and MBC was the average of three triplicates.

3.5.4. Determination of minimum fungicidal concentration of extract and fractions

Similar to MBC for standard bacterial species, Minimum Fungicidal Concentration (MFC) of 80% methanol extract and solvent fractions of plant extracts against fungal species was determined from those wells of plates which showed no visible growth in MIC assay. After selecting wells without visible growth or wells having concentration of extract/ fractions greater than or equal to MIC was streaked and sub-cultured into prepared PDA (antifungal free) medium in aseptic manner and incubated at 30 ºC (Arevalo *et al*., 2003; Mathur, 2013). So that, the highest dilution (least concentration) of plant extracts that yielded no single fungal colony growth was considered as MFC. The entire tests were performed in triplicate for both fungal species to ensure accuracy and the MFC was the average of three replicates.

3.6. Preliminary phytochemical screening

The qualitative phytochemical investigations of 80% methanol extract and each solvent fraction of *Urtica simensis* leaves were performed using standard tests as illustrated below.

Test for terpenoids

Two ml of chloroform was added into a test tube having 0.25 g of 80% methanol extract and each solvent fraction. Then, 3ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids (Shetty *et al*., 2016).

Test for saponins (frothing test)

Five ml of distilled water was added in a test tube having 0.25 g of 80% methanol extract and each solvent fraction. Then, the solution was shaken vigorously for 2-3 minutes and observed for a stable persistent froth. Formation of froth or foam indicated the presence of saponins (Nwadiaro *et al*., 2015).

Test for tannins (ferric chloride test)

About 0.25 g of 80% methanol extract and each solvent fraction was boiled in 10 ml of water in a test tube and then filtered. Three drops of 0.1% ferric chloride were added to the filtrate.

Presence of tannins was confirmed by the formation of brown greenish or blue-black color (Nwadiaro *et al*., 2015).

Test for flavonoids

Ten ml of ethyl acetate was added into a test tube having 0.25 g of 80% methanol extract and each solvent fraction, and heated on a water bath for 3 minutes. The mixture was cooled and filtered. Then, 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonial layer indicated the presence of flavonoids (Shetty *et al*., 2016).

Test for cardiac glycosides (keller kiliani test)

About 0.25 g of 80% methanol extract and each solvent fraction were diluted into 5 ml water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under lied with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the glacial acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Shetty *et al*., 2016).

Test for steroids

Two ml of acetic anhydride was added to 0.25 g of 80% methanol extract and each solvent fraction with 2 ml chloroform. Then, 1 ml of concentrated sulfuric acid was added. The color changed from violet to blue or green at the junction indicated the presence of steroids (Nwadiaro *et al*., 2015).

Test for alkaloids (Dragendroff reagent test)

About 0.5 g of 80% methanol extract and each solvent fraction were diluted to 10 ml of acid alcohol and stirred while heating in water bath for 10 minutes. Then the suspension was cooled, filtered and divided into two test tubes. To one test tube a few drops of Mayer's reagent was added, while to the other tube a few drops of Dragendorff's reagent was added. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids (Alhadi *et al*., 2015).

Test for anthroquinones

About 0.25 g of 80% methanol extract and each solvent fraction was shaken with 10 ml of benzene. This was filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet color in the ammonical (lower) phase indicated the presence of free hydroxyl anthroquinones (Nwadiaro *et al*., 2015).

Test for Phenols

About 0.25 g of 80% methanol extract and each solvent fraction was treated with few drops of 5% neutral ferric chloride solution; the appearance of a greenish color indicated the presence of phenols (Shetty *et al*., 2016).

3.9. Data analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA). The mean zone of inhibition of plant extracts and standard discs against each bacterial and fungal test organism was expressed as mean and standard error of mean (Mean ± SEM) of each three replicate. Mean zone of inhibition of different concentration of both 80% methanol extract and solvent fractions against one another and the standard discs were analyzed using one-way analysis of variance (ANOVA), and differences between means were determined by Post Hoc (Tukey) test at significant level set at $p \leq 0.05$. On top of that, regression analysis was followed to check the predictability of zone of inhibition as the concentration increase from 200mg/ml to 800 mg/ml so as R^2 values were calculated.

Since all experiments were conducted triplicate the MIC, MBC and MFC were analyzed as Mean ± SEM using descriptive statistics of SPSS.

4. RESULTS

4.1. Antibacterial activity

In this investigation, the antibacterial activities of both 80% methanol extract and each solvent fraction were evaluated using agar well diffusion method at concentration of 200, 400 and 800 mg/ml as shown in Table 1, 2 and 3. The concentration dependant R^2 predictive values were calculated for 80% methanol extract and each solvent fraction. Therefore, R^2 of 80% methanol extract ranged from 0.74 (*S. thypi*) to 0.96 (*S. flexneri*). For chloroform fraction R^2 values ranges from 0.79 (*S. pyogen*) to 0.93 (*S. aureus*). In addition the R^2 values of ethyl acetate fraction ranged from 0.88 (*S. pneumoniae*) to 0.95 (*K. pneumonia*) while n-butanol fraction R^2 values varied from 0.77 (*S. flexneri*) to 0.95 in *S.aureus* and *S. pyogen*.

Among the test bacteria, the maximum average zone of inhibition at 800 mg/ml concentration among gram positive bacterial species were 20.33 mm (*S. pneumonia*) followed by 20.00 mm and 19.33 mm for *S. aureus* and *S. pyogenes* respectively. On the other hand, the maximum average inhibition, at similar concentration in gram negative bacteria species were 19.00 mm (*K. pneumonia*) followed by 18.67 mm (*S. flexneri*) and 18.33 (*P. aeruginosa*). In contrary, no zone of inhibition was observed in 200 mg/ml of 80% methanol extract against *S.typhi*.

The mean comparisons of different concentration of extracts with each other and with the standard antibacterial discs were also presented in Table 1, 2 and 3. The average mean zone of inhibition of 80% methanol extract and each solvent fraction at 200 mg/ml was significantly different ($p<0.05$) compared to that of its zone of inhibition at 800 mg/ml against the growth of each test bacterium. Similarly, 200 mg/ml plant extract concentration had a significant difference ($p<0.05$) compared to 400 mg/ml for all bacterial species with the exception chloroform fraction (*S. pneumonia, E. coli,* and *S. flexneri*), 80% methanol extract (*S. flexneri* and *K. pneumonia*), n-butanol fraction (*E. coli*) and all solvent fractions against *S. typhi*.

In *E. coli* the ethyl acetate fraction and n-butanol fractions in *S. typhi*, 400 mg/ml concentration showed zone of inhibition significantly different from 800 mg/ml ($p<0.05$). Moreover, for the rest of test bacterial species, 400 mg/ml concentration was significantly different ($p<0.05$) from 800 mg/ml with the exception of 80% methanol extract (*S. aureus*, *P. aeruginosa* and *S.*

pneumonia), chloroform fraction (*S. pyogen* and *K. pneumonia*) and n-butanol fraction against (*P. aeruginosa* and *K. pneumonia*). In addition, zone of inhibition of different concentration of extracts (200, 400 and 800mg/ml) were significantly different ($p<0.05$) from their respective positive control discs.

Zone of inhibition of the ethyl acetate fraction at 400 mg/ml concentration was greater than that of crude extract at equal concentration, with a significant difference ($p<0.05$) against *S. typhi*, *P. aeruginosa*, *S. flexneri* and *K. pneumonia*. While, similar greater values of inhibition was observed in n-butanol fraction than the crude extract at equal concentration (400 mg/ml), with a significant difference ($p<0.05$) against *S. pneumoniae*, *S. typhi*, *S. flexneri* and *K. pneumonia.* In the opposite, the zone of inhibition of the crude extract was greater than that of the chloroform fraction at equal concentrations against *S. pneumoniae*, with a significant difference ($p<0.05$).

Similarly, zone of inhibition of n-butanol fraction (in gram positive bacteria) and ethyl acetate fraction (in gram negative bacteria) were greater than the crude extract at equal concentration (800 mg/ml), with a significant difference ($p<0.05$). Moreover, the zone of inhibition of the n-butanol fraction was greater than that of the crude extract at equal concentrations (800 mg/ml) against *S. typhi* and *S. flexneri*, with a significant difference ($p<0.05$) (Table 1, 2 and 3).

Higher antibacterial activities was shown in n-butanol fraction and followed by ethyl acetate fraction for gram positive *Streptococcus* species. But in *S. aureus,* the greater antibacterial activity in n- butanol fraction was followed by 80% methanol extract. While, the least activity among gram positive bacteria was recorded in chloroform fraction as shown in Table 1. On the other hand, in gram negative bacteria, higher antibacterial activity was shown in ethyl acetate fraction while least antibacterial activity was shown in chloroform fraction similar to gram positive bacteria with the exception of *S. typhi* and *P. aeruginosa* in which 80% methanol extract shown least activity as presented in Table 2 and 3. However, the aqueous extract didn’t show activity against any of bacterial species at any concentration.

Table 1: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram positive bacteria.

Test bacteria	Solvents & controls	Concentration of extracts			(+) control
		200 mg/ml	**400mg/ml**	**800mg/ml**	
S. aureus	80% methanol	11.00 ±0.58^{a3c2d3}	15.00±0.58^{a3}	17.33±0.67^{a3}	
	chloroform	10.67±0.33^{a3c2d3}	13.67±0.33^{a3d2}	16.67±0.33^{a3}	
	ethyl acetate	11.67±0.33^{a3c2d3}	14.33±0.33^{a3d2}	17.33±0.33^{a3}	
	n- butanol	13.00±0.00^{a3c3d3}	16.67±0.33^{a3d3}	20.00±0.00^{a3g2}	
	amoxicillin (30 µg/disc)				27.33±0.88
S. pyogenes	80% metahnol	9.67±0.67^{a3c2d3}	12.67±0.67^{a3d2}	16.00±0.00^{a3}	
	chloroform	8.67±0.67^{a3c2d3}	11.67±0.33^{a3}	13.67±0.67^{a3g2}	
	ethyl acetate	9.33±0.67^{a3c2d3}	13.67±0.67^{a3d2}	17.33±0.33^{a3}	
	n- butanol	10.67±0.33^{a3c2d3}	14.67±0.67^{a3d3}	19.33±0.33^{a3g2}	
	ampicillin (10 µg/disc)				28.00±0.00
S. pneumoniae	80% methanol	9.67±0.33^{a3c2d3}	13.00±0.00^{a3}	15.33±0.88^{a3}	
	chloroform	7.67±0.33^{a3d3}	9.33±0.67^{a3d2f3}	12.00±0.58^{a3g2}	
	ethyl acetate	10.67±0.33^{a3c3d3}	14.67±0.33^{a3d2}	17.33±0.33^{a3}	
	n- butanol	11.00±0.00^{a3c3d3}	16.33±0.33^{a3d3f2}	20.33±0.33^{a3g3}	
	ampicillin (10 µg/disc)				25.67±0.33

Values are expressed as mean ± SEM (n=3); The comparison of means of different concentration of extracts and control was analyzed using one way ANOVA followed by Tukey's Post hoc test; ^{a} compared with control value, ^{b} to 200mg/ml, ^{c} to 400mg/ml, ^{d} to 800mg/ml; ^{e} to crude 200mg/ml, ^{f} to crude 400mg/ml, ^{g} to crude 800mg/ml; $^{1}P<0.05$, $^{2}P<0.01$, $^{3}P<0.001$; (+) = positive control (ampicillin and amoxicillin).

Table 2: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram negative *E. coli*, *S. Typhi* and *P. aeruginosa* bacteria.

Test bacteria	**Solvent & controls**	**Concentration of extracts**			**(+) control**
		200 mg/ml	**400 mg/ml**	**800 mg/ml**	
E. coli	80% methanol	9.00 ±0.58^{a3c3d3}	12.33±0.33^{a3}	14.33±0.33^{a3}	
	chloroform	8.67±0.33^{a3d3}	10.00±0.58^{a3}	12.33±0.33^{a3}	
	ethyl acetate	11.00±0.00^{a3c2d3}	14.00±0.00^{a3d2}	17.33±0.33^{a3g2}	
	n- butanol	9.67±0.33^{a3d3}	11.00±0.00^{a3}	13.00±0.58^{a3}	
	amoxicillin (30 µg/disc)				27.00±0.00
S. typhi	80% methanol	NA	9.67±0.33^{a3}	12.00±0.00^{a3}	
	chloroform	8.33±0.33^{a3d3}	12.00±0.00^{a3}	14.33±0.33^{a3}	
	ethyl acetate	12.00±0.00^{a3d3}	15.00±0.00^{a3f3}	18.00±0.58^{a3g3}	
	n- butanol	10.33±0.33^{a3d2}	13.00±0.58^{a3d3f3}	17.00±0.58^{a3g3}	
	ciprofloxacin (5 µg/disc)				28.00±0.00
P. aeruginosa	80% methanol	8.67±0.33^{a3c2d3}	11.67±0.33^{a3}	14.00±0.58^{a3}	
	chloroform	9.00±0.58^{a3c2d3}	12.00±0.00^{a3d2}	15.00±0.58^{a3}	
	ethyl acetate	10.67±0.33^{a3c3d3}	15.00±0.58^{a3d2f2}	18.33±0.33^{a3g3}	
	n- butanol	8.67±0.33^{a3c2d3}	11.67±0.33^{a3}	14.33±0.00^{a3}	
	ciprofloxacin (5 µg/disc)				26.33±0.33

Values are expressed as mean ± SEM (n=3); The comparison of means of different concentration of extracts and control was analyzed using one way ANOVA followed by Tukey's Post hoc test; ^{a} compared with control value, ^{b} to 200mg/ml, ^{c} to 400mg/ml, ^{d} to 800mg/ml; ^{e} to crude 200mg/ml, ^{f} to crude 400mg/ml, ^{g} to crude 800mg/ml; $^{1}P<0.05$, ^{2} $P<0.01$, $^{3}P<0.001$. NA= no activity; (+) = positive control (amoxicillin and ciprofloxacin).

Table 3: Antibacterial activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against gram negative *S. flexneri* and *K. pneumonia* bacteria.

Test bacteria	**Solvent & controls**	**Concentration of extracts**			**(+) control**
		200 mg/ml	**400 mg/ml**	**800 mg/ml**	
S. flexneri	80% methanol	9.00 ± 0.58^{a3d3}	11.33 ± 0.33^{a3d2}	14.00 ± 0.00^{a3}	
	chloroform	11.00 ± 0.00^{a3d3}	13.33 ± 0.33^{a3d2}	16.00 ± 0.00^{a3}	
	ethyl acetate	11.00 ± 0.58^{a3c2d3}	15.00 ± 0.58^{a3d2f2}	18.67 ± 0.88^{a3g3}	
	n- butanol	11.00 ± 0.58^{a3c2d3}	14.33 ± 0.33^{a3d2f1}	17.67 ± 0.88^{a3g2}	
	ciprofloxacin (5 µg/disc)				27.00±0.00
K. pneumonia	80% methanol	10.33 ± 0.68^{a3d3}	12.33 ± 0.88^{a3d1}	15.33 ± 0.67^{a3}	
	chloroform	8.67 ± 0.88^{a3c3d3}	12.00 ± 0.00^{a3}	14.00 ± 0.00^{a3}	
	ethyl acetate	11.67 ± 0.33^{a3c2d3}	15.33 ± 0.33^{a3d3f2}	19.00 ± 0.00^{a1g2}	
	n- butanol	11.00 ± 0.58^{a3c2d3}	15.00 ± 0.00^{a3f2}	17.33 ± 0.67^{a2}	
	ciprofloxacin (5 µg/disc)				22.00±0.00

Values are expressed as mean ± SEM (n=3); The comparison of means of different concentration of extracts and control was analyzed using one way ANOVA followed by Tukey's Post hoc test; [a] compared with control value, [b] to 200mg/ml, [c] to 400mg/ml, [d] to 800mg/ml; [e] to crude 200mg/ml, [f] to crude 400mg/ml, [g] to crude 800mg/ml; $^{1}P<0.05$, $^{2}P<0.01$, $^{3}P<0.001$; (+) = positive control (ciprofloxacin).

4.2. Antifungal activity

The concentration dependant R^2 predictive values were calculated for 80% methanol extract and each solvent fraction against both fungal species. Hence, the R^2 values of 80% methanol extract were 0.80 and 0.93 in *A. niger* and *T. mentagrophytes* respectively. The R^2 values of chloroform fraction were 0.96 (*A. niger*) and 0.93 (*T. mentagrophytes*). Whereas, in ethyl acetate fraction R^2 predictive value of 0.81 (*A. niger*) and 0.89 (*T. mentagrophytes*). The respective R^2 values of n-butanol fraction for *A. niger* and *T. mentagrophytes* were of 0.88 and 0.90.

The mean zone of inhibition of all concentration of extracts showed significant difference ($p<0.05$) when compared with standard nystatin disc. Similarly, the zone of inhibition at 200 mg/ml for each extract was significantly different ($p<0.05$) compared to that of its zone of inhibition at 800 mg/ml against both test fungi. While, zone of inhibition of 200 mg/ml of each extract showed significant difference ($p<0.05$) when compared with 400 mg/ml only for 80% methanol extract against *A. niger* and ethyl acetate fraction against *T. mentagrophytes.* Moreover, the mean zone of inhibition of 400 mg/ml concentration was significantly different ($p<0.05$) from 800 mg/ml for chloroform fraction against both fungi, and in 80% methanol extract and ethyl acetate fraction against *T. mentagrophytes* as presented in Table 4.

Furthermore, zone of inhibition of the ethyl acetate fraction was greater than that of crude extract at equal concentration (200 mg/ml), with a significant difference ($p<0.05$) against *A. niger.* Similarly, at 400 mg/ml of equal concentration, zone of inhibition of the ethyl acetate fraction was greater than that of crude extract, with a significant difference ($p<0.05$) against both fungal species. But, the zone of inhibition was greater in crude extract than that of the chloroform fraction at equal concentration of 400 mg/ml, with a significant difference ($p<0.05$) against *A. niger*. Moreover, the ethyl acetate fraction against *T. mentagrophytes* also shown significantly greater zone of inhibition ($p<0.05$) when compared with crude extract at equal concentration of 800 mg/ml.

Additionally, ethyl acetate fraction showed better activity at all concentrations and maximum inhibition of 15.00 mm was observed at 800 mg/ml in both test fungi. Whereas, in *T. mentagrophytes* the 80% methanol extract, chloroform and n- butanol fractions were shown to have similar activity.

Table 4: Antifungal activities of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested fungi

Test fungi	Solvents & controls	Concentration of extracts			(+) control
		200mg/ml	400mg/ml	800mg/ml	
A.niger	80% methanol	7.67 ± 0.58^{a3c3d3}	11.67 ± 0.33^{a3}	13.33 ± 0.33^{a3}	
	chloroform	7.00 ± 0.00^{a3d3}	8.33 ± 0.33^{a3d3f3}	12.67 ± 0.33^{a3}	
	ethyl acetate	10.67 ± 0.33^{a3d3e3}	13.67 ± 0.67^{a3f1}	15.00 ± 0.00^{a3}	
	n- butanol	7.33 ± 0.33^{a3d3}	9.67 ± 0.00^{a3}	11.67 ± 0.33^{a3}	
	nystatin (100 units/ disc)				22.00±0.00
T. mentagrophytes	80% methanol	7.67 ± 0.67^{a3d3}	9.67 ± 0.33^{a3d2}	12.33 ± 0.33^{a3}	
	chloroform	7.33 ± 0.33^{a3d3}	9.67 ± 0.33^{a3d2}	12.00 ± 0.00^{a3}	
	ethyl acetate	8.33 ± 0.33^{a3c3d3}	12.67 ± 0.33^{a3f3d2}	15.00 ± 0.00^{a3g3}	
	n- butanol	8.00 ± 0.00^{a3d3}	10.33 ± 0.33^{a3}	12.00 ± 0.00^{a3}	
	nystatin (100 units/ disc)				24.00±0.00

Values are expressed as mean ± SEM (n=3); The comparison of means of different concentration of extracts and control was analyzed using one way ANOVA followed by Tukey's Post hoc test; [a] compared with control value, [b] to 200mg/ml, [c] to 400mg/ml, [d] to 800mg/ml; [e] to crude 200mg/ml, [f] to crude 400mg/ml, [g] to crude 800mg/ml; [1]$P<0.05$, [2] $P<0.01$, [3]$P<0.001$; (+) = positive control (nystatin).

4.3. The minimum inhibitory concentration of extracts against bacterial species

As shown in Table 5, the MIC value of 80% methanol extract and different solvent fractions were determined for extracts which has shown zone of inhibition in agar well diffusion test greater than or equal to 7 mm in diameter for all test bacteria species. Therefore, the MIC values extracts were in agreement with its preliminary antibacterial activities in agar well diffusion i.e. the more susceptible is the bacterium, the lower is the concentration of the extract required for growth inhibition in most of the test bacteria.

The MIC value of plant extracts against the tested bacteria ranged from 1.36 mg/ml (n-butanol fraction against *S. pneumoniae*) to 12.50 mg/ml (chloroform fraction against *E. coli*). The MIC values of 80% methanol extract ranged from 4.17 mg/ml (*S. pneumonia*) to 8.33 mg/ml (*K. pneumonia*) whereas in chloroform fraction it ranged from 4.17 mg/ml (*S. typhi* and *P. aeruginosa*) to 12.50 mg/ml (*E. coli*). While, a narrow range of MIC value was shown in ethyl acetate solvent fraction which ranged from 2.54 mg/ml (*K. pneumoniae*) to 5.21 mg/ml (*S. aureus*). Moreover, the MIC value of the n-butanol fraction was varied from 1.36 mg/ml of *S. pneumonia* to 6.25mg ml (*E. coli and P. aeruginosa*) as described in Table 5.

4.4. Minimum bactericidal concentration of extracts

Similar to MIC value, the MBC value of extracts, which were determined by sub-culturing the contents of wells having concentration of extract greater than or equal to MIC in prepared agar plate, were presented in Table 5. MBC values of the extracts against test organisms ranged from 5.21 mg/ml (ethyl acetate fraction against *E. coli* and n-butanol fraction against *S. pneumonia* and *S. aureus*) to 16.67 mg/ml (chloroform fraction against *E. coli* and 80% methanol fraction against *K. pneumonia*). In addition, 6.25 mg/ml (*S. pneumoniae*) and 5.21 mg/ml (*S. aureus*) were recorded as a minimum value for 80% methanol extract and n-buatnol fractions, respectively. On the other hand, the maximum MBC value in ethyl acetate fraction was 10.42 (*S. Typhi*). For chloroform fraction, the MBC value ranges from 6.25 mg/ml (*P. aeruginosa*) to 16.67 mg/ml in *E .coli*.

In general, the n-butanol and ethyl acetate fractions were more potent and killed gram positive and gram negative bacteria respectively, at lower concentration compared to that of the 80% methanol extract and other solvent fractions.

Table 5: MIC and MBC in (mg/ml) of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested bacterial species.

Solvents	**Concentration of extracts**	**bacterial test species**			
		S. aureus	***E .coli***	***P. aeruginosa***	***S. typhi***
80% methanol	MIC (mg/ml)	6.25±0.00	6.25±0.00	6.25±0.00	6.25±0.00
	MBC(mg/ml)	6.25±0.00	12.50±0.00	10.42±2.08	12.50±0.00
Chloroform	MIC (mg/ml)	8.33±2.10	12.50±0.00	4.17±1.04	4.17±1.04
	MBC(mg/ml)	12.50±0.00	16.67±4.12	6.25±0.00	12.50±0.00
Ethyl acetate	MIC (mg/ml)	5.21±1.04	3.13±0.00	2.60±0.52	2.60±0.52
	MBC(mg/ml)	8.33±2.80	5.21±1.04	6.25±0.00	6.25±0.00
n-butanol	MIC (mg/ml)	2.60±0.52	6.25±0.00	5.20±1.04	3.13±0.00
	MBC(mg/ml)	5.21±1.04	8.33±2.10	10.42±2.08	12.50±0.00
		S. flexneri	***S. pyogen***	***K. pneumoniae***	***S. pneumoniae***
80% methanol	MIC (mg/ml)	5.21±1.04	5.21±1.04	6.25±0.00	4.17±1.04
	MBC(mg/ml)	8.33±2.08	12.5±0.00	10.42±2.08	6.25±0.00
Chloroform	MIC (mg/ml)	6.25±0.00	6.25±0.00	8.33±2.08	6.25±0.00
	MBC(mg/ml)	10.42±2.08	8.33±2.08	16.67±4.12	8.33±2.08
Ethyl acetate	MIC (mg/ml)	2.60±0.52	3.13±0.00	2.54±0.59	3.13±0.00
	MBC(mg/ml)	6.25±0.00	6.25±0.00	6.25±0.00	6.25±0.00
n-Butanol	MIC (mg/ml)	3.13±0.00	2.60±0.52	3.13±0.00	1.36±0.00
	MBC(mg/ml)	6.25±0.00	6.25±0.00	6.25±0.00	5.21±1.04

All values are mean ± SEM of three replicates (n=3) as analyzed by SPSS; MIC=Minimal inhibitory concentration, MBC= minimum bactericidal concentration

4.5. The minimum inhibitory/ fungicidal concentration of extract against fungi

The MIC and MFC value ranging in between the concentration of 3.13 to 16.67 mg/ml (MIC) and 6.25 to 20.83 mg/ml (MFC) against fungal species as presented in Table 6. Ethyl acetate fraction was the most potent against *A. niger* in which its MIC and MFC value are 3.13 and 6.25 mg/ml respectively. The ethyl acetate fraction also induced growth inhibition and fungicidal effect at 4.17 and 6.25 mg/ml respectively on *T. mentagraphyte* fungi. On the other hand, the respective MIC and MFC values of n-butanol fraction against *A. niger* were 8.33 and 12.5 mg/ml. The lower antifungal activity was observed in chloroform fraction against *T. mentagraphyte* with MIC values of 10.42 mg/ml and MFC of 20.83 mg/ml.

Finally, this MIC and MFC values were in correspondence with the activity of plant extract in zone of inhibition test against *A. niger*. In contrary, even though 80% methanol extract, chloroform and n-butanol fraction showed similar activity in agar well diffusion test against *T. mentagraphyte* their corresponding MIC and MFC values greatly vary as presented in Table 6.

Table 6: MIC and MFC in (mg/ml) of 80% methanol extract and solvent fractions of the leaves of *Urtica simensis* against tested fungal species.

Solvents	**Concentration of extract**	**fungal species**	
		A. niger	***T. mentagraphyte***
80% mathanol	MIC (mg/ml)	4.17±1.04	6.25±0.00
	MFC(mg/ml)	10.42±2.08	16.67±4.17
Chloroform	MIC (mg/ml)	6.25±0.00	10.42±2.08
	MFC(mg/ml)	10.42±2.08	20.83±4.17
Ethyl acetate	MIC (mg/ml)	3.13±0.00	4.17±1.04
	MFC(mg/ml)	6.25±0.00	6.25±0.00
n-butanol	MIC (mg/ml)	8.33±2.08	6.25±0.00
	MFC(mg/ml)	12.50±0.00	12.50±0.00

All values are mean ± SEM of three replicates (n=3) as analyzed by SPSS. Where, MIC=Minimal inhibitory concentration, MFC= minimum fungicidal concentration

4.6. Preliminary phytochemical screening

Evaluation of the preliminary phytochemical screening of the 80% methanol extract of the leaves of *Urtica simensis* plant revealed the presence of terpenoids, saponins, tannins, flavonoids, steroids, alkaloids and phenols. Tannins were detected in both 80% methanol extract and each solvent fraction, except aqueous fraction. While, anthraquinones and glycosides were absent in each extracts. Moreover, ethyl acetate and n-butanol fractions held tannins, terpenoids and alkaloids in common. Additionally, ethyl acetate fraction revealed saponins and phenols which were not found in n-butanol fraction. Flavonoids and steroids only found in n-butanol fraction but not in ethyl acetate fraction. Chloroform fraction revealed tannins, steroids and phenols. Amongst all fractions, the ethyl acetate and n-butanol fractions appeared to be relatively rich in secondary metabolites while, the aqueous fraction held only saponins as shown from shown in Table 7.

Table 7: Preliminary phytochemical screening of the 80% methanol extract and solvent fractions of the leaves of *Urtica simensis*.

Secondary metabolites	**Crude extract**	**Solvent fractions**			
	80% methanol extract	**chloroform fraction**	**ethyl acetate fraction**	**n-butanol fraction**	**aqueous fraction**
Terpenoids	+	_	+	+	_
Saponins	+	_	+	_	+
Tannins	+	+	+	+	_
Flavonoids	+	_	_	+	_
Cardiac glycosides	_	_	_	_	_
Steroids	+	+	_	+	_
Alkaloids	+	_	+	+	_
Anthraquinones	_	_	_	_	_
Phenols	+	+	+	_	_

+ = present and - = absent

5. DISCUSSION

Wide spread development of resistance to currently available antimicrobials and undesirable side effect of those synthetic drugs in the treatment of infectious diseases, encourage the study of medicinal plants and plant derived compounds. Therefore, this study was performed to investigate the antimicrobial activity of the leaves of *Urtica simensis* 80% methanol extract and solvent fractions on some pathogenic bacterial and fungal species.

In this study plant, the n-butanol fraction and ethyl acetate fractions showed greater activity in the majority of test organisms when compared with other solvent fractions and 80% methanol extract. This finding was consistence with the finding of Uzair *et al* (2016) in the *Iris germinica* plant antimicrobial activities in which the n-butanol and ethyl acetate fractions shown greater activity than other organic solvents. This might be due to the higher concentration of bioactive secondary metabolites in these fractions.

Furthermore, the n-butanol fraction showed greater antibacterial activity against gram positive bacterial test organisms (*S. aureus* and *Streptococcus* species). This greater activity of n-butanol fraction in gram positive bacteria might be attributed to the presence of secondary metabolite like flavonoids and steroids as the mixture of these two bioactive compounds had more activity against gram positive bacteria as described by (Taleb-Contini *et al*., 2003).

The plant extract/ fractions showed relatively higher zone inhibition in gram positive bacteria than most of gram negative bacteria and fungi at similar concentrations. This might be due to higher activity of the extract/ fractions on gram positive bacteria as most plant extract/ fractions were more active in gram positive bacteria (Wintola and Afolayan, 2015; Rabe and Staden, 1997). The reason of higher activity of extracts in gram positive bacteria might be associated with the cell wall structure in these groups of bacteria as it is easily penetrated due to the presence of none efficient peptidoglycan layer (Kuiuar and Schweizer, 2005).

On the other hand, the ethyl acetate fraction of the extract showed better activity against gram negative bacterial and fungal test organisms when compared with other the solvent fraction and 80% methanol extract. The higher activity of ethyl acetate fraction in gram negative organisms might be associated with the presence of phenolic metabolite additional to terpenoids, saponins, tannins and alkaloids. Therefore, the higher activity of ethyl acetate fraction in these groups of

organisms might be associated with the detection of phenolic compounds in this fraction since phenol possesses the ability to form complex in gram negative bacteria outer membrane (lipo-polysaccharide layer) which act as a strong barrier (Smith-Palme et al., 1998).

The higher activity of ethyl acetate fraction in fungal species might be associated with the presence of secondary metabolites like terpenoids, saponins, tannins, alkaloids and phenols in ethyl acetate fraction with antifungal activity and their synergy (Beatriz *et al*., 2012; Mohanta *et al*., 2007; Saxena *et al*., 2013).

In the majority of test microorganisms, the n- butanol and ethyl acetate fractions showed better activity. The higher activity of these two fractions might be associated with the number of bioactive metabolites and their synergetic activities. In the opposite, low activity of chloroform fraction might be due to the presence of lower quantity of metabolites detected. In addition, low activity of chloroform fraction might be due to the polar nature of the plant bioactive constituents with antimicrobial activity as chloroform was found to be the best solvent for the extraction of non-polar biological active compounds (Harmala *et al*., 1992).

The leaves of *Urtica simensis* 80% methanol extract and solvent fractions showed variation in antimicrobial activity. These variations in the activity of extract/ fractions in this particular study plant might be attributed to the difference in level of solubility of components of *Urtica simensis* plant active ingredients in various solvents (Shafique *et al*., 2011). Moreover, the study plant showed antibacterial activities against gram positive and gram negative bacteria as well as the *Urtica simensis* also had antifungal activities against both tested fungal species, these is an indicative of the presence of broad spectrum bioactive metabolites in the study plant (Srinivasan *et al*., 2001).

All plant extracts tested in this study showed antimicrobial activities against bacterial and fungal test organisms, except aqueous fraction which showed no activity against any microorganisms regardless of the tested concentrations used. This might be because of more active organic compounds were extracted in the organic solvents and not in water as the plant had been extracted in sequential solvents of increasing polarity (Tiwari *et al*, 2011). In addition, the absence of antibacterial activity of the aqueous fraction might be associated with the absence of almost all of the secondary metabolites tested with the exception of saponins in this fraction, as

displayed in the preliminary phytochemical screening test (Table 7). Even though, saponin was found in aqueous fraction, this fraction was devoid of antimicrobial activity, the reason might be associated with low concentration of saponins in this fraction.

Moreover, all plant extracts with the exception of aqueous fraction showed dose dependent increased inhibitory activity as concentration was increased from 200 to 800 mg/ml; this proportionality increase in inhibitory activity (zone of inhibition) as the concentration of extract increase were also reported in other studies on other plants (Cyriacus and Eberechukwu, 2013; Judaki *et al*., 2014; Shetty *et al*., 2016). This relationship between the concentrations of extracts and zones of inhibition might be because of more extract/fractions were able to diffuse into the agar media so as the concentration of active metabolites also increase, and inducing more inhibition of microbial growth.

Even though, the test plant having antimicrobial activity at all concentrations of crude and solvent fractions, except 80% methanol extract didn't show any activity against *S. typhi* at 200 mg/ml concentration, the mean zone of inhibition of all test concentrations against bacterial and fungal test organisms were not statistically comparable to that of their respective positive control discs ($p < 0.05$). This might be due to the less concentration of the active metabolites in the 80% methanol extract and solvent fractions than standard antimicrobial discs.

Moreover, for 80% methanol extract and each solvent fraction, the antimicrobial activities at concentration of 200 mg/ml were significantly different from 800 mg/ml ($p < 0.05$). This might be due to the fact that as the concentration of extracts increase, the concentration of active metabolites deposited to the well also increases, as described by Kowti *et al* (2010) in similar study.

Zone of inhibition of plant extract/fractions against susceptible bacteria and fungi were inversely proportional to their MIC, MBC and MFC values. That is, the more susceptible the organisms to the extract/fractions in agar well diffusion method, the less is its corresponding MIC, MBC and MFC values. In other word, the greater inhibition zone of extract/fractions in agar well diffusion method was corresponding with smaller values of extract/fractions in (mg/ml) required to inhibit or kill organisms, this suggest the reproducibility of the experiments.

However, slight discrepancies were observed in 80% methanol extract and ethyl acetate fraction against *S. aureus*. The inconsistency also seen in *T. mentagrophyte* where the 80% methanol extract, chloroform and n-butanol fractions showed almost similar zone of inhibition but their respective MIC, MBC and MFC greatly varies. This might be associated with the more sensitivity of micro-dilution methods to determine the antimicrobial activity of plant extract/fractions, as describe by Scorzoni *et al* (2007).

In this particular study, the MIC value of the active plant extract/fractions obtained were lower than MBC and MFC values except 80% methanol fraction against *S. aureus* with equal MIC and MBC values, suggesting that the plant extracts were bacteriostatic/fungistatic at lower concentration but bactericidal/fungicidal at higher concentration (Delahaye *et al*., 2009; Ige *et al*., 2015).

The present study also revealed that the plant extracts had bactericidal property because all the extract using different solvent were shown to have MBC/MIC ratios ranges from 1 (in 80% methanol fraction against *S. aureus*) to 3.99 (n-butanol frication against *S. typhi*), as an agent is considered to be bactericidal when the ratio of MBC/MIC ranged between 1:1 to 4:1(French, 2006). In fungal test organisms, most of plant extract showed fungicidal activity, except n-butanol fraction against *A. niger* and chloroform fraction against *T. mentagraphyte* which showed fungistatic activity because an agent considered to be fungicidal when the ratio of MFC/MIC ranges between 1:1 and 2:1 (Ige *et al*., 2015).

The need for phytochemical screening has become imperative, since many plants accumulate biologically active, complex organic chemicals (secondary plant metabolites) in their tissues. In the present study, the preliminary phytochemical screening of the 80% methanol extract of *Urtica simensis* leaves revealed the presence of terpenoids, saponins, tannins, flavonoids, steroids, alkaloids and phenols, and these phyto-constituents could be responsible for the significant *in vitro* antibacterial and antifungal activities of *Urtica simensis*.

Although the exact mechanism of the antimicrobial action of 80% methanol extract and each solvent fraction of *Urtica simensis* could not be established in this study, a number of investigators have shown that constituents such as alkaloids, tannins, saponins, phenols, terpenoids and flavonoids in general are responsible for antimicrobial action of different plant

extract/fractions through different mechanisms (Aboaba and Efuwape, 2001; Bonjar *et al*., 2004; Shetty *et al*., 2016). However, the antimicrobial properties of these extract could not be attributed to anthraquinones and cardiac glycosides which were both absent in this extract.

Tannin was detected in the 80% methanol extract and all solvent fractions except, in aqueous fraction which was devoid of tannins. Different workers have shown that tannins have antibacterial and antifungal activity and has the ability to inhibit the growth of pathogenic bacteria and fungi (Pranoothi *et al*., 2014; Wink, 2015), suggesting the extended inhibition of bacteria and fungi in all solvent extracts positive for tannins in the current study might be due to the presence of this groups of bioactive secondary metabolites. As described in different studies, the possible mechanism of antibacterial and antifungal action of tannins may include binding to protein adhesins and promotes inhibition of enzymes which in turn leads to rupture of the plasma membrane and deprivation of the microbial substrate (Pereira *et al*., 2015), leads to cell death (Mailoa *et al*., 2014).

Terpenoids are another bioactive compound detected from the 80% methanol extract, ethyl acetate and n-butanol fractions of plant. The presence of terpenoids especially in the ethyl acetate and n-butanol fractions might be associated with higher antibacterial and antifungal activities since terpenoid compounds isolated from different plants shown to have antifungal (Beatriz *et al*., 2012; Shai *et al*., 2008), and antibacterial activities (Bama *et al*., 2012). The possible antimicrobial inhibition might be associated with its action on cell membrane disruption and mitochondrial dysfunction (Bama *et al*., 2012; Freiesleben and Jager, 2014).

Similar to terpenoids, the alkaloids bioactive compound was found from 80% methanol extract, ethyl acetate and n-butanol fractions of plant extract, so as better activity ethyl acetate and n-butanol fraction in majority of test microorganisms might be associated with the presence of terpenoids and alkaloids in both solvents. As described by many researchers on medicinal plants, alkaloids have antibacterial and antifungal activities (Saxena *et al*., 2013; Singh and Kumar, 2011). The possible mechanism of action includes interfering with DNA topoisomerase enzyme, cytoskeleton structure and/or protein biosynthesis machinery (Wink, 2015).

On the other hand, flavoniods were found in 80% methanol extract and only n-butanol fraction. As illustrated by different researcher, flavonoids isolated from ginger (*Zingiber officinale*

Roscoe) and garlic (*Allium sativum* L.) had antibacterial effect (Ekwenye and Elegalam, 2005), while flavonoids isolated from stem bark of *Erythrina burtii* had antifungal activity (De Campos *et al*., 2005), this study on different plants shown that flavoniods have both antibacterial and antifungal activities. The possible mechanism of action of flavoniods includes the precipitation of proteins of the cell and inhibits the synthesis of nucleic acid (Anandhi *et al*., 2014).

Furthermore, the *Urtica simensis* plant leaves also contain saponins bioactive metabolites particularly in 80% methanol extract, ethyl acetate and aqueous fractions. As described by Mohanta *et al* (2007) and Saxena *et al* (2013) in different time, saponins have multiple effects against bacterial and fungal pathogens. The possible mechanism of inhibition might be associated with its binding to cell membrane of the cell and leads to severe tension of the membrane and leakage of macromolecules and ions out of the cell (Netala *et al*., 2015; Wink, 2015).

Finally, phenols and steroids also qualitatively detected from the study plant. The antimicrobial activities of this study plant extract might be associated with the presence of steroids in 80% methanol extract, chloroform and n-butanol fractions. Moreover, a phenols compound were also detected in 80% methanol extract, chloroform and ethyl acetate fractions, and contributes the antimicrobial activities of study plant. As explicated in different studies, the presences of phenols and steroids bioactive metabolites in medicinal plants have shown antibacterial and antifungal properties (Ansari *et al* 2013; Cowan, 1999; Karou *et al*., 2005). The possible mechanism of antimicrobial activities of phenolic metabolites might be either by substrate deprivation or membrane disruption (Peres *et al*., 1997). On the other hand, the antimicrobial inhibition of steroidal compound might be accompanied by its association with membrane lipid and causing leakages from liposomes (Epand, 2007).

In general the antimicrobial activity of bioactive compounds detected from *Urtica simensis* may leads to death of bacterial and fungal test organisms through various mechanisms like membrane disruption, inhibition of enzymes, and deprivation of the microbial substrate, mitochondrial dysfunction, inhibit the synthesis of nucleic acid. Therefore, the overall antimicrobial effect of plant extracts might due to the presence of concentrated bioactive compounds or due to synergistic activity of two or more active metabolites.

6. CONCLUSION

The results of the present study revealed that the *Urtica simensis* leaves are endowed with antimicrobial activity. With the exception of the aqueous extracts, the 80% methanol extract and the other solvent fractions showed different levels of antimicrobial activity on the tested bacterial and fungal species. Some of these fractions were more effective than the others depending up on the species of the organism. Among the solvent fractions, n-butanol fraction being the most active fraction in gram positive bacteria, while ethyl acetate fraction is most active fraction in gram negative bacterial and fungal test organisms. But aqueous fraction is devoid of any activity against any of test organisms. These activities may be attributed to the presence of bioactive secondary metabolites including flavonoids, tannins, terpenoids, saponins, phenols, steroids and alkaloids that act either individually or collectively to bring about the overall antimicrobial effect. Semi-polar and non-polar constituents found in ethyl acetate, n-butanol and chloroform fractions may have better antimicrobial activity against test organisms, while highly polar constituents found in the aqueous fraction is without antimicrobial activity. The antimicrobial activity exhibited by the plant extracts against the pathogenic test organisms used in this study provide a scientific support for the traditional use of the *Urtica simensis* leaves as treatment of wound infection caused by bacterial and fungal pathogens.

7. RECOMENDATIONS

Based on the findings of the present study, the following recommendations are suggested to further investigate the experimental plant in depth.

- Further extensive and elaborated studies are required to isolate, purify and identify pharmacologically active principle (s) responsible for the antimicrobial activity of the plant.
- The mechanism of action for this plant active ingredient should be elaborated in detail.
- In *vivo* antimicrobial studies of the crude and each solvent fraction should be conducted to confirm effectiveness of the plant against bacterial and fungal pathogens *in vitro*.
- The antimicrobial activities of this plant should also be done in other bacterial and fungal species which were not dealt in this study.

8. REFERENCE

Abdullah FE, Khan MI, Waheed S (2013). Current pattern of antibiotic resistance of clinical isolates among conjunctival swabs. *Pak J Med Sci* **29**: 81–84.

Aboaba O and Efuwape BM (2001). Antibacterial properties of some Nigerian species. *Biol Res Commun* **13**: 183–188.

Acharyya S, Patra A, Bag PK (2009). Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant *Vibrio cholerae*. *Trop J Pharm Res* **8**: 231–237.

Aderiye BI and Oluwole OA (2015). Antifungal agents that target fungal cell wall components: A review. *Agric Biol Sci J* **1**: 206–216.

Alemayehu K, Asfaw Z, Kelbessa E (2015). Ethnobotanical study of medicinal plants used by local communities of Minjar-Shenkora District, North Shewa Zone of Amhara Region, Ethiopia. *J Med Plant Studies* **3**: 1–11.

Alhadi EA, Khalid HS, Alhassan MS, Kabbashi AS, Noo MO (2015). Antimicrobial and phytochemical screening of *Cordia africana* in Sudan. *World J Pharm Res* **4**: 257–269.

Alsan MM, Westerhaus M, Herce M, Nakashima K, Farmer PE (2011). Poverty, global health and infectious disease: Lessons from Haiti and Rwanda. *Infect Dis Clin North Am* **25**: 611–622.

Altinyay C, Eryılmaz M, Yazgan AN, Yılmaz B, Altun ML (2015). Antimicrobial activity of some *Alnus* species. *Eur Rev Med Pharmacol Sci* **19**: 4671–4674.

Aly HIM, Salem MZM, Gohar YM, El-Sayed AB, Ashmawy NA (2012). Efficacy of methanolic extract and its fractions of some tree species for potential in vitro antibacterial activity. *Int J Agric Food Res* **1**: 12–27.

Amin MM, Sawhney SS, Jassal M (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale* Wudpecker. *J Pharm Pharmocol* **2**: 1–5.

Anandhi D, Srinivasan PT, Kumar JP, Jagatheesh S (2014). DNA fragmentation induced by the glycosides and flavonoids from *C.coriaria*. *Int J Curr Microbiol App Sci* **3**: 666–673.

Anderson RJ, Groundwater PW, Todd A, Worsley AJ (2012). Antibacterial agents: Chemistry, mode of action, mechanisms of resistance and clinical applications. A John Wiley & Sons, Ltd., Publication. United Kingdom.

Annapurna SM (2015). Traditional applications and phytochemical investigations of *Lonicera japonica.* Thunb. *Int J Drug Dev Res* **7**: 42–49.

Ansari MA, Anurag A, Fatima Z, Hameed S (2013). Natural phenolic compounds: A potential antifungal agent. Microbial pathogens and strategies for combating them: Science, technology and education. *FORMATEX*: 1189–1195.

Ararsa A and Bekele A (2015). Assessment of self-medication practice and drug storage on private pharmacy clients in Jimma town, Oromia, South west Ethiopia. *AJPS* **1**: 20–32.

Arevalo MP, Carrillo-Munoz A, Salgado J, Cardenes D, Brio S, Quindos G, Espinel-Ingroff A (2003). Antifungal activity of the echinocandin anidulafungin (VER002, LY-303366) against yeast pathogens: a comparative study with M27-A micro-dilution method. *J Antimicrob Chemother* **51**: 163–166.

Arif T, Bhosale JD, Kumar N (2009). Natural products: Antifungal agents derived from plants. *J Asian Nat Prod Res* **11**: 621–638.

Bajracharya AM, Yami KD, Prasai T, Basnyat SR, Lekhak, B (2008). Screening of some medicinal plants used in Nepalese traditional medicine against enteric bacteria. *Sci World* **6**: 107–110.

Balouiri M, Sadiki P, Ibnsouda SK (2016). Methods for in vitro evaluating antimicrobial activity: A review. *JPA* **6**: 71–79.

Bama S, Kingsley SJ, Sankaranarayanan S, Bama P (2012). Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens* linn.: Identification and mode of action of the terpenoid compound as antibacterial. *Int J Pharm Pharm Sci* **4**: 557–564.

Barsi DF and Fan SH (2005). The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian J Pharmacol* **37**: 26–29.

Beatriz p, Ezequie V, Azucena O, Pilar C (2012). Antifungal activity of *Psidium guajava* organic extracts against dermatophytic fungi. *J Med Plant Res* **41**: 5435–5438.

Belakhdar G, Benjouad A, Abdennebi EH (2015). Determination of some bioactive chemical constituents from *Thesium humile* Vahl. *J Mater Environ Sci* **6**: 2778–2783.

Bellamy R (2004). Susceptibility to infectious diseases:The importance of host genetics. Cambridge University Press, New York.

Benezie IFF and Galor SW (2011). Herbal medicine: Biomolecular and clinical aspects, 2nd (ed), Taylor & Francis Group. London.

Bernhoft A (2010). A brief review on bioactive compounds in plants. In: Bioactive compounds in plants benefits and risks for man and animals (Aksel Bernhoft ed.) Proceedings from a symposium held at the Norwegian Academy of Science and Letters, November 13–14 2008, Oslo, Norway.

Bonjar GHS, Nik AK, Aghighi S (2004). Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of South East regions of Iran. *J Biol Sci* **4**: 405–412.

Buller N, Thomas A, Barton M (2014). Antimicrobial Susceptibility Testing. Australia and New Zealand standard diagnostic procedures: 1-30. [Online] available at: https://www. google. com/url?sa [Accessed on October 5, 2016].

CDC (2011). A CDC framework for preventing infectious diseases: Sustaining the Essentials and Innovating for the Future. Atlanta, Georgia. [Online] Available at: http://www.cdc.gov/ drugresistance/threat-report-2013/ [accessed on Septmber 25, 2015].

Chekole G, Asfaw Z, Kelbessa E (2015). Ethnobotanical study of medicinal plants in the environs of Tara-gedam and Amba remnant forests of Libo Kemkem District, northwest Ethiopia. *J Ethnobiol Ethnomed* **11**: 1–38.

Chukwudi US and Mohammed HI (2013). Phytochemical screening and antibacterial activity of the fruit and leaf extracts of *Tamarindus Indica* (Linn.). *Rep opin* **5**: 18–28.

CLSI (2014). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational Supplement. M100-S24. Document. CLSI, USA. **34**: 220–230.

Conly J (2002). Antimicrobial resistance in Canada. *CMAJ* **167**: 885–891.

Cowan MM (1999). Plant products as antimicrobial agents. *Clin Microbiol Rev* **12**: 564–582.

Cyriacus OI and Eberechukwu AF (2013). Antimicrobial activity of ethanol extract of four indigenous plants from south eastern Nigeria. *ARPN J Sci Technol* **3**: 350–355.

Cyrus WG, Daniel GW, Nanyingi MO, Njonge FK, Mbaria JM (2008). Antibacterial and cytotoxic activity of Kenyan medicinal plants. *Mem Inst Oswaldo Cruz* **103**: 650–652.

Daoowd WS (2013). In vitro antifungal activity of extracts of Anastatica hierochuntica. J Vet Med Sci 4: 142–148.

De Campos MP, Chechinel Filho V, Da Silva RZ, Yunes RA, Zacchino S, Juarez S, Bella Cruz RC, Cruz AB (2005). Evaluation of antifungal activity of *Piper solmsianum* C. DC. var. solmsianum (Piperaceae). *Biol Pharm Bull* **28**: 1527–1530.

Deepa P, Chaithanneya R, Ramabhat P (2015). Phytochemical properties and antimicrobial activities of leaf, bark, fruit extracts and silver nanoparticles of *Samadera indica* Gaertner. *Eur J Biotechnol Biosci* **3**: 30–37.

Delahaye C, Rainford L, Nicholson A, Mitchell S, Lindo J, Ahmad M (2009). Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. *J Med Biol Sci* **3**: 1–7.

Dereje A, Tegene N, Adugn T (2016): Methane concentration, organic matter digestibility, metabolisable energy and short chain fatty acid production of morphological fractions of stinging nettle (*Urtica simensis*) measured through an in vitro gas test. *Global Veterinaria* **16**: 276–284.

Dey SK, Banerjee D, Chattapadhyay S, Karmak KB (2010). Antimicrobial activities of some medicinal plants of west Bengal. *Int J Pharm Pharm Sci* **1**: 1–9.

Dhanalakshmi D and Manimegalai K (2013). Antibacterial activity of leaf and seed extracts of *Delonix regia* and *Achyranthus aspera* against selected bacterial strains. *Int J Pharm Med Bio* Sc **2**: 30–35.

Dyubeni L and Buwa LV (2012). An ethnobotanical study of plants used for the treatment of ear, nose and throat (ENT) infections in Nkonkobe Municipality, South Africa. *J Med Plants Res* **6:** 2721–2726.

Ekwenye UN, Elegalam NN (2005). Antibacterial activity of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts on *Escherichia coli* and *Salmonella typhi*. *Int J Mol Med Adv Sci* **1**: 411–416.

Enyew A, Asfaw Z, Kelbessa E, Nagappan R (2014). ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. *Curr Res J Biol Sci* **6**: 154–167.

Epand RF (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. *Biochimica et Biophysica Acta* **1768**: 2500–2509.

Erenso F and Maryo M (2014). Endemic plant species composition and their status in Boda dry evergreen montane forest, West Showa, Ethiopia. *Int J Biodivers Conserv* **6**: 563–569.

Eskedar GA, Gulelat DH, Getachew AD (2013). Nutritional profile of *Samma* (*Urtica simensis* Steudel) leaves grown in Ethiopia. *IJSID* **366**: 454–461.

Fauci AS and Morens DM (2012). The perpetual challenge of infectious diseases. *N Engl J Med* **366**: 454–461.

Fodouop SPC, Simo RT, Amvene JM, Talla E, Etet PFS, Takam p, Kamdje AHN, Muller J (2015). Bioactivity and therapeutic potential of plant extracts in cancer and infectious diseases. *J Dis Med Plants* **1**: 8–18.

Freiesleben SH and Jager AK (2014). Correlation between plant secondary metabolites and their antifungal mechanisms. A review. *Med Aromat Plant* **3**: 2–6.

French GL (2006). Bactericidal agents in the treatment of MRSA infections:the potential role of daptomycin. *J Antimicrob Chemother* **58**: 1107–1117.

Gebrehiwot K and Hundera K (2014). Species composition, plant Community structure and natural regeneration status of Belete Moist Evergreen Montane Forest, Oromia Regional state, southwestern Ethiopia. *MEJS* **6**: 97–101.

Gebrezgabiher G, Kalayou S, Sahle S (2013). An ethno-veterinary survey of medicinal plants in woredas of Tigray region, Northern Ethiopia. *Int J Biodivers Conserv* **5**: 89–97.

Ghannoum MA and Rice LB (1999). Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin Microbiol Rev* **12**:501–517.

Grublwn GJH and Denton OA (2004). Plant resource of tropical Africa 2: Vegetables. 541–543. PROTA Foundation, Wageningen, Netherland.

Gulcin I, Kufrevio OI, Oktay M, Buyukokuroglu ME (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J Ethnopharm* **90**: 205–215.

Harmala P, Vuorela H, Tornquist K, Hiltunen R (1992). Choice of solvent in the extraction of *Angelica archangelica* roots with reference to calcium blocking activity. *Plant Med* **58**: 176–183.

Hessen MT and Kaye D (2004). Principles of use of antibacterial agents. *Infect Dis Clin N Am* **18**: 435–450.

Hospenth DR and Rinaldi MG (2015). Diagnosis and treatment of fungal infections. 2nd (ed). Springer International Publishing. Switzerland.

Ige AO, Mebude OO, Adeniyi BA, Adewoye EO (2015). Antidermatophytic activities of *Musa sapientum* methanol leaf extract in-vitro. *BMRJ* **10**: 1–7.

Jampilek J (2016). Potential of agricultural fungicides for antifungal drug discovery. *Expert Opin Drug Discov* **11**:1–9.

Judaki A, Panahi J, Havasian MR, Tajbakhsh P, Roozegar MA (2014). Study of the inhibitory effect of *Quercus Coccifera's* aqueous extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa In vitro*. *Bioinformation* **10**: 689–692.

Kanafani ZR and Perfect JR (2008). Resistance to antifungal agents: mechanisms and clinical impact. *Clin Infect Dis* **46**: 120–128.

Karou D, Dicko MH, Simpore J, Traore AS (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *Afr J Biotechnol* **4**: 823–828.

Kavalali GM (2003). *Urtica*: Therapeutic and nutritional aspects of stinging nettles. Taylor & Francis Lrd. London.

Kawsud P, Puripattanavong J, Teanpaisan R (2014). Screening for anticandidal and antibiofilm activity of some herbs in Thailand. *Trop J Pharm Res* **13**: 1495–1501.

Kefalew A, Asfaw Z, Kelbessa E (2015). Ethnobotany of medicinal plants in Ada'a District, East Shewa Zone of Oromia Regional State, Ethiopia. *J Ethnobiol Ethnomed* **11**:1–28.

Khalifa RA, Nasser MS, Goma AA, Osman NM, Salem HS (2013). Resazurin microtiter assay plate method for detection of susceptibility of multidrug resistant *Mycobacterium tuberculosis* to second-line anti-tuberculous drugs. *EJCDT* **62**: 241–247.

Koenig KL and Schultz CH (2014). The 2014 Ebola Virus Outbreak and Other Emerging Infectious Diseases. USA.

Kowti R, Harsha R, Ahmed MG, Hareesh AR, Gowda T, Dinesha R, Kumar SBP, Ali IM (2010). Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata* P. Beauv. *Res J Pharm Biol Chem Sci* **11**: 961–969.

Kuiuar A and Schweizer HP (2005). Bacterial resistance to antibiotics: active eftiux and reduced uptake. *Adv Drug Deliv Rev* **57**: 1486–1513.

Kumar JR, Muralidharan S, Parasuraman S (2014). Antifungal agents: New approach for novel delivery systems. *J Pharm Sci Res* **6**: 229–235.

Kuta FA, Tsado DJ, Garba SA, Saidu AN (2015). Antibacterial activity of the leaf and stem bark crude extracts of Khaya senegalensis. *Med Aromat Plant Res J* **3**: 9–15.

Liwa AC and Jaka H (2015). Antimicrobial resistance: Mechanisms of action of antimicrobial agents. The battle against microbial pathogens: Basic Science, Technological Advances and Educational Programs. *FORMATEX*: 876–885.

Mabberley DJ (2008). The Plant Book 3rd (ed), Cambridge University Press, Cambridge.

Mabeku LBK (2011). Screening of some plants used in the Cameroonian folk medicine for the treatment of infectious diseases. *Int J Biol* **3**: 13–22.

Maertens JA and Marr KA (2007). Diagnosis of fungal infections. Informa healthcare USA, Inc. USA.

Mahesh B and Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J Agric Sci* **4**: 839–843.

Mailoa MN., Mahendradatta M, Laga A, Djide N (2014). Antimicrobial activities of tannins extract from guava leaves (*Psidium Guajava* L) on pathogens microbial. *Int J Sci Technol Res* **3**: 236–241.

Mainous AG and Pomeroy C (2010). Management of antimicrobials in infectious diseases: Impact of antibiotic resistance. 2nd (ed). Springer Science+Business Media. New York, USA.

Mandell GL, Douglas RG, Bennett JE (2005). Principles and practice of infectious diseases, 6th (ed.) Churchill Livingstone, An Imprint of Elsevier, USA.

Maregesi SM and Mwakalukwa R (2015). Ethnopharmacological study on medicinal plants used to treat infectious diseases in the Rungwe District, Tanzania. *Int J Med Plant Nat Prod* **1**: 15–22.

Maroyi A (2013). Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. *J Ethnobiol Ethnomed* **9**: 31–49.

Maryo M, Nemomissa S, Bekele T (2015). An ethnobotanical study of medicinal plants of the Kembatta ethnic group in Enset-based agricultural landscape of Kembatta Tembaro (KT) Zone, Southern Ethiopia. *Asian J plant Sci Res* **5**: 42–61.

Mathur R (2013). Phytochemical and antimicrobial evaluation of plant extracts of *Enicostemma hyssopifolium*. *J Pharmacogn Phytochem* **2**: 30–36.

Mehta KC, Dargad RR, Borade DM, Swami OC (2014). Burden of antibiotic resistance in common infectious diseases: Role of antibiotic combination therapy. *J Clin Diagn Res* **8**: 1–8.

Mendelson M (2015). Role of antibiotic stewardship in extendingthe age of modern medicine. *S Afr Med J* **105**: 414–418.

Mohanta TK, Pare JK, Pal DK, Thatoi HN (2007). Evaluation & antimicrobial activity and phytochemical screening ot oils and nuL & *Semicarpus anacardium*. *Le Sci Res Ess* **2**: 486–490.

Morens DM, Folkers GK, Fauci AS (2004). The challenge of emerging and re-emerging infectious diseases. *Nature* **430**: 242–249.

Nair R and Chanda SV (2007). Antibacterial activities of some medicinal plants of Western Region of India. *Turk J biol* **31**: 231–236.

Nanuta P and Mukeslr R (2012). Medicinal plants used as antimicrobial agents: a review. *IRJP* **3**: 31–41.

Ndamane Y, Kambizi L, Songca SP, Oluwafemi OS (2013). Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system. *J Med Plant Res* **7**: 2755–2760.

Netala VR, Ghosh SB, Bobbu P, Anitha D, Tartte V (2015). Triterpenoid saponins: a review on biosynthesis, applications and mechanism of their action. *Int J Pharm Pharm Sci* **7**: 24–28.

Njume C and Goduka NI (2012). Treatment of diarrhoea in rural African communities: An overview of measures to maximize the medicinal potentials of indigenous plants. *Int J Environ Res Public Health* **9**: 3911–3933.

Nomura R, Nakano K, Nemoto H, Fujita K, Inagaki S, Takahashi T, Taniguchi K, Takeda M, Yoshioka H, Amano A, Ooshima T (2006). Isolation and characterization of *Streptococcus mutans* in heart valve and dental plaque specimens from a patient with infective endocarditis. *J Med Microbio* **55**: 1135–1140.

Nwadiaro PO, Ogbonna AI, Wuyep PA, Sila-Gyang MD (2015). Antifungal activity of *Cucumis metuliferus* E.Mey. ex Naudin on some post-harvest decay fungi of string beans. *JAIR* **3**: 490–496.

Paramythiotou E, Frantzeskaki F, Flevari A, Armaganidis A, Dimopoulos G (2014). Invasive fungal infections in the ICU: How to approach, How to treat. *Molecules* **19**: 1085–1119.

Paul S, Emran TB, Saha D, Hosen SM Z (2012): Phytochemical and antimicrobial activity of the plant extracts of *Brassica oleracea* against selected microbes. *Asian J Pharm Med Sci* **2**: 40–45.

Pereira AV, Santana GM, Gois MB, Sant-Ana DMG (2015).Tannins obtained from medicinal plants extracts against pathogens: antimicrobial potential. The battle against microbial pathogens: basic science, technological advances and educational programs. *FORMATEX:* 224–242.

Peres MTP, Monache FD, Cruz AB, Pizzolatti MG, Yunes RA (1997). Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). *J Ethnopharmacol* **56**: 223–226.

Perlin DS, Shor E, Zhao Y (2015). Update on antifungal drug resistance. *Curr Clin Microbiol Rep* **2**: 84–95.

Powers JH (2004). Antimicrobial drug development the past, the present, and the future. *Clin Microbiol Infect* **10**: 23–31.

Pranoothi EK, Narendra K, Joshi DSS, Swathi J, Sowjanya KM, Rathnakarreddi KVN, Emmanuel RFE, Padmavathi C, Satya AK (2014). Studies on qualitative, quantitative, phytochemical analysis and screening of in vitro biological activities of *Leucas indica* (L) VAR. Nagalapuramiana. *Int J Herbal Med* **2**: 30–36.

Prasad P and Ramakrishnan N (2012). Chromatographic finger print analysis of *Rumex vesicarius* L. by HPTLC Technique. *Asian Pac J Trop Biomed* **1**: 1–7.

Rabe T and Staden J (1997). Antibacterial activity of South African plants used for medicinal purposes. *J Ethanopharmacol* 56: 81–87.

Radhika SM and Michael A (2013).In vitro antifungal activity of leaf extracts of *Azadirachta indica*. *Int J Pharm Sci* **5**: 723–725.

Radwan IN, Randa B, Hend AN, Camilia G (2015). Evaluation of antimicrobial efficacy of four medicinal plants extracts used as root canal irrigant on *Enterococcus faecalis*: An in-vitro study. *Int Dent Med J Adv Res* **1**: 1–8.

Ramtin M, Massiha A, Khoshkholgh-Pahlaviani MRM, Issazadeh K, Assmar M, Zarrabi S (2014). In vitro antimicrobial activity of *Iris pseudacorus* and *Urtica dioica*. Zahedan J Res Med Sci **16**: 35–59.

Rasheed JK, and Tenover FC (2003). Detection and characterization of antimicrobial resistance genes in bacteria. *Manual Clin Microbiol* **75**: 1196–1212.

Reta H, Asfaw Z, Kelbessa E (2015). Contribution of traditional farmers for medicinal plant conservation on the farming site in Gozamin District, Amhara Region, Ethiopia. *Int J Life Sci* **4**: 24–35.

Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013). Phytochemistry of medicinal plants. J *Pharmacogn Phytochem* **1**: 168–182.

Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Mendes-Giannini MJS (2007). Comparative study of disk diffusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts *Candida* spp and *Cryptococcus* sp. *J Basic Appl Pharma Sci* **28**: 25–34.

Sen A and Batra A (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach*. *Int J Curr Pharm Res* **4**: 67–74.

Shafique S, Shafique S, Bajwa R, Akhtar N, Hanif S (2011). Fungitoxic activity of aqueous and organic solvent extracts of *Tagetes erectus* on phytopathogenic fungus *Ascochyta rabiei*. *Pak J Bot* **43**: 59–64.

Shaik G, Sujatha N, Mehar SK (2014). Medicinal plants as source of antibacterial agents to counter *Klebsiella pneumonia*. *J Appl Pharm Sci* **4**: 135–147.

Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN (2008). Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. *J Ethnopharmacol* **119**: 238–244.

Sharma S, Ali SA, Khare A, Nailwal TK (2015); Genetic diversity analysis of *Urtica Parviflora* in Uttarakh and Himalayas by rapid marker. *J Biotechnol Biomater* **5**: 1-4.

Shetty SB, Mahin-Syed-Ismail P, Varghese S, Thomas-George B, Kandathil-Thajuraj P, Baby D, Haleem S, Sreedhar S, Devang-Divakar D (2016). Antimicrobial effects of *Citrus sinensis* peel extracts against dental caries bacteria: An in vitro study. *J Clin Exp Dent* **8**: 70–77.

Shrestha P, Adhikari S, Lamichhane B, Shrestha BG (2015). Phytochemical screening of the medicinal plants of Nepal. *JESTFT* **1**:11–17.

Singh R, Kumari N, Gangwar M, Nath G (2015). Qualitative characterization of phytochemicals and in vitro antimicrobial evaluation of leaf extract of *Couroupita guianensis* AubL. A threatened medicinal tree. *Int J Pharm Pharm Sci* **7**: 212–215.

Smith-Palmer A, Stewart J, Fyfe L (1998). Antimicrobial properties of plant essential oils and essences against five important foodborne pathogens. *Lett Appl Microbiol* **26**: 118–122.

Sosa AJ, Byarugaba DK, Amabile-Cuevas CF, Hsueh P, Kariuki SK, Okeke AN (2010). Antimicrobial resistance in developing countries. Springer Science and Business Media. New York, USA.

Srinivasan D, Perumalsamy LP, Nathan S, Sures T (2001). Antimicrobial activity of certain Indian medicinalplants used in folkloric medicine. *J Ethnopharm* **94**: 217–222.

Taleb-Contini SH, Salvador MJ, Watanabe E, Ito IY, Oliveira DC (2003). Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. *Braz J Pharm Sci* **39**: 404–408.

Taye B, Giday G, Animut A, Seid J (2011). Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pac J Trop Biomed* **2**: 370–375.

Taylor J, Hafner M, Yerushalmi E, Smith R, Bellasio J, Vardavas R, Bienkowska-Gibbs T, Rubin J (2014). Estimating the economic costs of antimicrobial resistance: Model and Results. Wellcome Trust, Cambridge, UK.

Tenover FC (2006). Mechanisms of antimicrobial resistance in bacteria. *Am J Med* **119**: 3–10.

Thakur S and Sharma DR (2015). Antibacterial activity and high performance liquid chromatography (HPLC) analysis of *Asparagus adscendens* Roxb. root extracts. *Int J Adva Sci Technl Res* **5**: 359–371.

Tiwari P, Kumar B, Kaur N, Kaur G, Kaur H (2011). Phytochemical screening and extraction: A review. *Int Pharm Sci* **1**: 98–106.

Tura BK, Kibebew WH, Admassu AM (2014). Investigating the role of apiculture in watershed management and income improvement in Galessa protected area, Ethiopia. *Agric Forest Fish* **3**: 380–385.

Uzair A, Bakht J, Iqbal A, Naveed K, Ali N (2016). *In vitro* antimicrobial activities of different solvent extracted samples from *Iris germinica*. *Pak J Pharm Sci* **29**: 145–150.

Van ND and Tap N (2008). An overview of the use of plants and animals in traditional medicine systems in Viet Nam. A traffic South East Asia report. Ha Noi, Viet Nam.

Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R (2013). Phytochemical analysis of medicinal plants occurring in local area of mardan. *Biochem Anal Biochem* **2**: 1–4.

WHO (2012). Global report for research on infectious disease of poverty. World Health Organization. Geneva, Switzerland. [Online] available at: http://www.who.int/tdr/stewardship/global_report/Summary_advocacy_web.pdf [accessed on November 03, 2016].

WHO (2013). Research priorities for the environment, agriculture and infectious diseases of poverty. World Health Organization, Switzerland, Geneva. [Online] available at: http://www.who.int/mediacentre/factsheets/fs310/en/index2.html [accessed on o 18, 2016].

WHO (2014). Antimicrobial resistance: Global report on surveillance. WHO, Geneva, Switzerland. [Online] available at: http://www.who.int/drugresistance/documents/AMR_report_Web_slide_set.pdf?ua=1 [accessed on November 18, 2016].

Wink M (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines* **2**: 251-286.

Wintola OA and Afolayan AJ (2015).The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora Africana* Thunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complement Altern Med* **15**: 1–12.

Wu ZY, Milne RI, Chen CJ, Liu J, Wang H, Li DZ (2015): Ancestral state reconstruction reveals rampant homoplasy of diagnostic morphological characters in urticaceae, conflicting with current classification schemes. The university of Edinburgh. [Online] available at: http://dx.doi.org/10.1371/journal.pone.0141821 [accessed on November 18, 2016].