IN VITRO ANTIMICROBIAL ACTIVITY OF "ANTIBACT" AND ITS COMPONENT PLANTS AGAINST CLINICAL BACTERIAL ISOLATES.

By

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

This work is dedicated to God almighty without whom I am nothing. Secondly, to my entire family especially my father, Mr C. U Igbukolu, siblings; Henrietta, Oge and John Igbukolu for their encouragement, invaluable support, prayer, and love.

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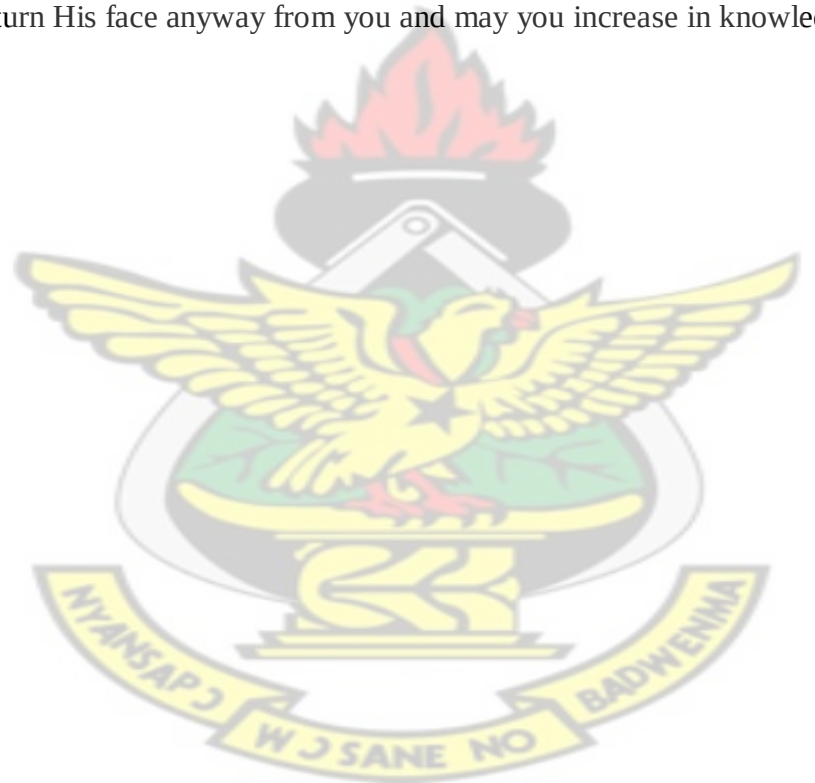

ABSTRACT

Plants used for traditional medicine contain a wide range of substances which can be used to treat various infectious diseases. Hence, ethanol and water extracts from the following plants were analyzed: *Psidiumguajava*(guava), *Cymbopogoncitratus* (lemongrass), *Hoslundiaopposita, Phyllanthusfraternus*and a formulation, "Antibact" comprising of a combination of the above mentioned plants. These plants were selected based on existing traditional medicine knowledge, usage and their potential as antimicrobial agents. The antimicrobial activity of the plant extracts were evaluated against twenty one antibiotic susceptible and resistant bacterial strains using agar-well diffusion method. In addition, the possible *in vivo* toxic effect of "Antibact" was studied.

Phytochemical screening showed that the herbal medicinal extracts under study contained saponins, reducing sugars, phenolics, polyuronides, triterpenes, alkaloids flavonoids, and phytosterols. The antibacterial activity was more in ethanol extracts compared to aqueous extract in all the plants indicating that the active compounds responsible for antibacterial activity is more soluble in organic solvents. The highest antimicrobial potentials were observed for the extracts of *P. guajava*inhibiting 57% and 71.4% of the microorganisms used for aqueous and ethanolic extracts respectively, 57% and 48% for *P. fraternus*followed by *H. opposita*inhibiting 24% and 14.3% while *C. citratus* extracts did not present any antimicrobial activity. Preparations made from the plant extracts were active against some of thecontrol strains of bacteria used in this study; *P. mirabilis* ATCC 49565, *P. aeruginosa*ATCC 27853, *S. aureus*ATCC 25922, *S. saprophyticus*ATCC 15305, *S. typhi* ATCC 19430 and *S. typhimurium*ATCC 14028. The most resistant species were *E. coli, K.*

pneumoniae, S. typhi, and *S. typhimurium*being resistant to all the plant extracts used in this study. The ethanol extract of "Antibact" was more effective than the aqueous extract inhibiting the growth of thirteen out of twenty one (62%) microbes used while the aqueous "Antibact" inhibited the growth of a total of five out of twenty one (23.8%) microorganisms used with an average zone of 2.32 ± 0.93mm and 6.68 ± 1.26mm respectively. Gram-negative bacteria were less sensitive than Gram-positive bacteria, which may be due to their differences in the cell wall composition. The low MICs observed ranging from 0.5 to 32.0mg/ml during the study indicate that these herbal preparations are efficacious and can be used for the management of diseases caused by the test organisms.

Results obtained support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new medicines. Further study should involve investigating the potential of additional medicinal plant(s) which are known to inhibit the growth of Gram negative bacteria in order to make "Antibact" more effective, investigation of the phytochemical present in "Antibact" responsible for the antibacterial effect in this study and testing of efficacy of "Antibact" using laboratory animals such as mice, guinea pigs and rabbits.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Herbalism also known as botanical medicine, medical herbalism, herbal medicine, herbology or phytotherapy, is a traditional medicinal (TM) or folk medicine practice based on the use of plants and plant extracts. A medicinal plant, as defined by World Health Organization (WHO), is a plant in which some or all of its parts can be used directly in the management of a disease (Acharya and Shrivastava, 2008). Sofowora (1982), similarly, defines medicinal plant as a plant in which one or more of its organs contain substances that can be used for therapeutic purposes. Herbal medicine may be made of cellular structures such as leaf, bark, petal, root or may be made of non-cellular structural agents such as gum, latex and others (Malau *et al*., 2009). Herbal medicine may also be a decoction, which may be in cold water or prepared by bringing it to boil and allowing it to cool, or tisane, which is tea made by either decoction, or infusion (Malau *et al*., 2009). The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts (Falodun, 2010).

In the last decade, there has been a global upsurge in the use of traditional medicine (TM) and complementary and alternative medicines (CAM) in both developed and developing countries. TM/CAM is a group of diverse medical and health care systems, practices, and products including acupuncture, herbalism, traditional Chinese medicine, naturopathy, Ayurveda, hypnosis, and homeopathy in addition to a range of other practices that are not generally considered to be part of conventional medicine and are not integrated into the dominant health care system in most

countries (WHO, 2005). Today, traditional, complementary and alternative medicines play an increasingly important role in health care and health sector reform globally. Hence, the safety and efficacy, as well as the quality control, of TM and CAM have become important concerns for both health authorities and the public (WHO, 2005).

In Ghana, Kenya and Mali, research has shown that a course of pyrimethamine/sulfadoxine antimalarials can cost several dollars (Ahorlu *et al.*, 1997). Yet per capita out-of-pocket health expenditure in Ghana and Kenya amounts to only around $6 per year, thus, most populations simply cannot afford orthodox drugs (WHO, 2002). Conversely, herbal medicines for treating malaria are considerably cheaper and may sometimes even be paid for in kind and/or according to the "wealth" of the client. TM is highly popular in many developing countries because it is firmly embedded within wider belief systems (*WHO, 2002) and i*t is also a major African socio-cultural heritage, that was once believed to be primitive and wrongly challenged with animosity by foreign religions and conventional or orthodox medical practitioners (Malau *et al*., 2009). In recent years, however, natural products are of worldwide economic interest because drug resistance shown by disease organisms is on the increase (White and Nosten, 1993) and herbal remedies are being sought by a cross section of scientist for various ailments (Malau *et al*., 2009).

WHO (2000) has estimated conservatively that, between 60 and 90 percent of the population of the non-industrialized countries rely on medicinal plants to meet their health care needs, either totally or partially. With the scarcity of Doctors and paucity

of hospitals and clinics, the large majority of most populations have to rely on sources other than allopathic medicine for their health care (www.crvp.org/book/Series02/II-5/chapter_vii.htm). According to Boateng and Darko (2008), in a survey within the business district of Accra, it came out clear that the average Ghanaian on the street had at least an idea on what herbal medicine is with eight out of every ten randomly selected respondents believing that herbal medicine is of great importance in contemporary Ghanaian health system (Darko, 2009). Fewer Clinics and Hospitals operating in rural areas, the distance villagers have to travel to get to the nearest Health Care Centers, the 'cash and carry' system that operate in most Clinics and Hospitals, the delay in getting a consultation with a Doctor and the high cost of drugs which are hardly available in the first place, are some of the reasons accounting for the popularity of herbal medicine practice in Ghana (Mshana *et al*., 2000). Thus, several problems have made herbal medical practice once again popular, and while the influence of orthodox medicine in many developing countries is declining, most people now patronize the service of herbal medicine.

Antimicrobials of plant origin have enormous therapeutic potentials to heal many infectious diseases and are mostly not associated with serious side effects (Iwu *et al*., 1999). Hence, the potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicines to act against pathogenic microbes. "Antibact" is a formulation – an antimicrobial herbal product from the Centre for Scientific Research into plant medicine (CSRPM) Mampong – Akwapim. "Antibact" consist of four medicinal plants (*Psidium guajava* (guava), *Cymbopogon citratus* (lemongrass), *Hoslundia opposita, Phyllanthus fraternus*)

which are known to possess antimicrobial properties. Different parts of these plants are used in the indigenous system of medicine for the treatment of various human ailments ranging from sores, cold, skin diseases, veneral diseases, fever, stomach upset to various other painful spasmodic affections of the intestines.

1.2 PROBLEM STATEMENT

Numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance, probably emanating from selective pressure of antimicrobial usage (Oskay *et al.*, 2009). Currently, there is growing interest in exploiting plants for medicinal purposes especially in Africa and this may stem from the fact that microorganisms are developing resistance to many drugs and this has created a situation where some of the common and less expensive conventional antimicrobial agents are losing their efficacy (Montefiore *et al*., 1989).

1.3 JUSTIFICATION FOR THE STUDY

Over one-third of the population in developing countries lack access to essential medicines and therefore the provision of safe and effective traditional medicine/Complementary alternative medicine (TM/CAM) therapies could, become a critical tool to increase access to health care (WHO, 2002).

1.4. OBJECTIVES OF THE RESEARCH

1.4.1. Aim

The main aim of this study is to "Evaluate the efficacy and safety of 'Antibact', an antimicrobial herbal product from the Centre for Scientific Research into Plant medicine (CSRPM), Mampong-Akwapim.

1.4.2 Specific objectives

1. To phytochemically screen 'Antibact' and its component plants
2. To determine the antibiogram of the microbes used
3. To screen "Antibact" and its component plants for antimicrobial activity against wild and standard microbes
4. To determine the minimum inhibitory concentrations (MICs) of "Antibact"
5. To determine the minimum bacteriocidal concentrations (MBCs) of "Antibact"
6. To determine the safety of 'Antibact' using experimental animals

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. HERBAL MEDICINE

Many studies have been conducted to investigate the phytochemical constituents and antimicrobial activity of medicinal plants with the intention of finding more effective treatment (both topical and systemic applications) as alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant (Mills-Robertson, 2004; Akinpelu and Onakoya, 2006; Mills-Robertson *et al*., 2009). During the last ten years, the pace of development of new orthodox antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple resistance) has increased astronomically with the increase in number of antibiotic resistant bacteria no longer being matched by the expansion in the arsenal of agents available to treat infections (Akinpelu and Onakoya, 2006). Literature reports and ethnobotanical records suggest plants as the sleeping giants of pharmaceutical industry (Hostettmann and Hamburger, 1991). These medicinal plants may provide natural sources of antimicrobial drugs that could provide novel or lead compounds that may be employed in controlling some infections globally (Akinpelu and Onakoya, 2006). Unfortunately, this component of ethnomedicine has long been ignored by many biomedical practitioners for various reasons. For example, the chemical composition, dosages, and toxicity of the plants used in ethnomedicine are not clearly defined (Lowe *et al*., 2000). However, it is interesting to note that the ethnomedicinal uses of plants is one of the most successful criteria used by the pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine (Williams, 2006).

Even though in Africa phytomedicine has been in existence for hundreds of years even before the colonial administration and is still in use today with about 80% of the population depending on herbal medicine for its primary health care delivery, the introduction of allopathic/orthodox medicine in the form of base chemicals and pharmaceuticals during the 18th and 19th centuries demonstrated method for bringing quick relief from sufferings and this won instant admiration and popularity (Elujoba *et al.*, 2005; Okigbo and Mmeka, 2006). However, in the 1980's the revival of interest in natural drugs started due to the dangers of overmedication or harmful side effects of synthetic drugs (Sunita *et al.*, 2010). Furthermore, there is a widespread belief that 'green' medicine is healthier than synthetic products (Sunita *et al.*, 2010). Consequently, between 1995 and 2000, the number of doctors who had undergone special training in natural remedy medicine almost doubled worldwide (Sunita *et al.*, 2010).

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Zhang, 2010). Regulation of exploitation and exportation is therefore essential, together with international cooperation and coordination for their conservation so as to ensure their availability for the future (Zhang, 2010). Despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been studied for possible medical applications (Zhang, 2010). Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparations containing them (Zhang, 2010).

2.2. ANTIBIOTIC RESISTANCE AND INFECTIOUS DISEASES

Antibiotics are used extensively for treating diseases caused by bacteria, yet these biological weapons do not always accomplish their mission. Antibiotics resistance is the ability of a microorganism to withstand the effects of an antibiotic. This resistance may develop through gene action or plasmid exchange between bacteria of the same species (WHO, 2011). If a bacterium carries several resistant genes, it is called multiresistant or, as it is often described, a 'superbug' (WHO, 2011). In the early 1970s, physicians were forced to abandon their belief that, given the vast array of effective antimicrobial agents, virtually all bacterial infections were treatable (Lowy, 2003). Their optimism was shaken by the emergence of resistance to multiple antibiotics among such pathogens as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis* (Lowy, 2003). Resistance to antibiotics is a serious worldwide problem which is increasing and has implications for morbidity, mortality, and health-care both in hospitals and in the community (Franco *et al*., 2009). The irony of this trend toward progressively more resistant bacteria is that it coincides with a period of dramatically increased understanding of the molecular mechanisms of antimicrobial resistance (Lowy, 2003). Unfortunately, while this insight has resulted in the identification of novel drug targets in herbal medicine, it has not yet resulted in effective new chemotherapeutic agents (Lowy, 2003). Several studies have demonstrated that, patterns of antibiotic usage can have dramatic effects on the prevalence of resistant organisms (WHO, 2011). Irrational use of antibiotics in humans and animal species, insufficient patient education when antibiotics are prescribed, lack of guidelines for treatment and control of infections, inadequate dissemination of scientific information for physicians on the rational use of antibiotics, and lack of official

government policy on the rational use of antibiotics in public and private hospitals, have all contributed to antibiotic resistance (Franco *et al.*, 2009).

Infectious diseases account for one half of all deaths in tropical countries irrespective of efforts made in controlling the incidence of epidemics (Okigbo and Ajalie, 2005). It is on record that, of the 39.5 million deaths in the developing countries, 9.2 million is estimated to have been caused by infectious and parasitic diseases (Hart and Kariuki, 1998). Infections of the lower respiratory tract formed the third most common cause of death worldwide, with diarrhoeal diseases being fourth. In children, it is estimated that, ninety eight per cent of deaths occur in the developing world, and that most of these deaths occur as a result of infections with bacteria constituting an important cause of morbidity and mortality in human beings all over the world (Hart and Kariuki, 1998).

For decades, antimicrobial drugs have proven useful for the treatment of bacterial infections but lately most bacteria are inherently resistant to newly developed antimicrobial agents (Newman *et al.*, 2006). The emergence of acquired resistance to antimicrobial drugs had been observed in almost all pathogenic bacteria (Newman *et al.*, 2006). The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases (Mathias *et al.*, 2000; Gibbons, 2005). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some cases all, effective antibiotics (Mills-Robertson *et al.*, 2002; 2003a; 2003b; Kapil, 2005). Furthermore changing patterns of susceptibility and the availability of new antimicrobial agents require continuous updating of knowledge

concerning treatment of diseases caused by such pathogens (Gottlieb *et al.*, 2002). Thus, there is the need to look for alternative strategies for the management of disease resistant bacteria and one of the possible strategies towards this objective involves rational localization of bioactive phytochemicals which have antibacterial activity and this may be one of the important approaches for the containment of antibiotic resistance (Gottlieb *et al.*, 2002).

According to Dubey *et al.*, (2004), the potential of higher plants as a source for new drugs is still largely unexplored. For instance, traditional medicine is currently being used in the treatment and care of such life-threatening illnesses as malaria and AIDS. In Ghana, Mali, Nigeria and Zambia, herbal medicines are the first line treatment for more than 60% of children with high fever and studies in Africa and North America have shown that approximately 75% of people living with HIV/AIDS use traditional medicine alone or in combination with other medicines for various symptoms or conditions (*WHO, 2003).* Today plants are the almost exclusive source of medicines for the majority of the worlds' population with most populations in the developing countries utilizing traditional medicine for their primary health care needs (http://www.crvp.org/book/Series02/II-5/chapter_vii.htm).

In Ghana, antibiotics are available to the public from a variety of sources, including hospitals and pharmacies; licensed chemical sellers and drugstores; markets and roadside stalls and hawkers. They are commonly purchased without a prescription, even though this practice is illegal. This widespread availability has led to the inappropriate use by patients and health care providers, and consequently, a steady increase in drug resistance **(**ADMER, 2010). The increase in resistance is costing

money, livelihoods, and lives and threatens to undermine the effectiveness of the country's health delivery programs **(**ADMER, 2010**).** There is also inadequate information in Ghana on the susceptibility of microorganisms to antimicrobial agents used for treatment of diseases (Newman *et al*., 2006).

The severe disease burden caused by resistant pathogens is highlighted by a recent study in Tanzania of outcomes of bloodstream infections in children (Blomberg *et al.*, 2007). The incidence of bloodstream infections was 14% in 1,818 children less than 7 years of age (Blomberg *et al.*, 2007). One third (35%) of these children died, with 44% of patients having Gram-negative bloodstream infections, which were more than twice the mortality from malaria (Blomberg *et al*., 2007). Inappropriate treatment resulting from antimicrobial resistance was a significant risk factor for the mortality; resistance rates to ampicillin/amoxicillin in *E. coli* were around 90%, and those drugs were the most frequently prescribed (Blomberg *et al.*, 2007). This illustrates the grave consequences of antibiotic resistance to the burden of diseases in low-income countries.

Understanding the mechanisms and the spread of antimicrobial resistance is an important step in curtailing the problem, and it may also allow pharmaceutical companies to develop new drugs that foil common resistance mechanisms (Nester *et al*., 2007).

2.3 MECHANISMS OF ANTIBIOTIC RESISTANCE

Bacteria can resist the effects of antimicrobials through a variety of mechanisms (Nester *et al*., 2007). Some species of bacteria are innately resistant to ≥1 class of

antimicrobial agents (Tenover, 2006). In some cases this resistance is innate, but in many others it is acquired (Nester *et al*., 2007). Of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent (Tenover, 2006). Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera. Some of these mechanisms include;

2.3.1 Drug-inactivating Enzyme

The organism may acquire genes encoding enzymes, such as □-lactamases, that destroy the antibacterial agent before it can have an effect (Tenover, 2006). Another example is the enzyme chloramphenicol acetyltransferase which chemically alters the antibiotic chloramphenicol (Nester *et al*., 2007).

2.3.2 Increased Elimination of the Drug

The systems that bacteria use to transport detrimental compounds out of a cell are called efflux pumps (Nester *et al*., 2007). Bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect (Tenover, 2006).

2.3.3 Alternation in the Target Molecule

An antimicrobial drug generally acts by recognizing and binding to a specific target molecule in a bacterium. Minor structural changes in the target can prevent the drug from binding (Nester *et al*., 2007). Bacteria may acquire several genes for a

metabolic pathway which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site via down regulation of porin genes (Tenover, 2006).

2.4 ACQUISITION OF RESISTANCE

Antimicrobial resistance can be due to either spontaneous mutation (*vertical evolution*), which alters existing genes, or acquisition of new genes (*horizontal evolution*) which may occur between strains of the same species or between different bacterial species or genera (Nester *et al*., 2007).

2.4.1 Spontaneous Mutation

As cells replicate, spontaneous mutation occur at a relatively low rate. Even at a low rate, however, such mutation can ultimately have a profound effect in the resistance of a bacterial population on an antimicrobial drug (Nester *et al*., 2007). Spontaneous mutations may cause resistance by the following;

- Altering the target protein to which the antibacterial agent binds by modifying or eliminating the binding site (e.g., change in penicillin-binding protein 2b in pneumococci, which results in penicillin resistance),
- Upregulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in staphylococci),
- Downregulating or altering an outer membrane protein channel that the drug requires for cell entry (e.g., OmpF in *E coli*), or

- Upregulating pumps that expel the drug from the cell (efflux of fluoroquinolones in *S aureus*)

In all of these cases, strains of bacteria carrying resistance-conferring mutations are selected by antimicrobial use, which kills the susceptible strains but allows the newly resistant strains to survive and grow (Tenover, 2006).

2.4.2 Gene Transfer

Mechanisms of genetic exchange include conjugation, transduction, and transformation. Each of these processes, transposons may facilitate the transfer and incorporation of the acquired resistance genes into the host's genome or into plasmids. During conjugation, a gram-negative bacterium transfers plasmid-containing resistance genes to an adjacent bacterium, often via an elongated proteinaceous structure termed a *pilus*, which joins the 2 organisms (Tenover, 2006). Conjugation among gram-positive bacteria is usually initiated by production of sex pheromones by the mating pair, which facilitate the clumping of donor and recipient organisms, allowing the exchange of DNA. During transduction, resistance genes are transferred from 1 bacterium to another via bacteriophage (bacterial viruses). This is now thought to be a relatively rare event. Finally, transformation, i.e., the process whereby bacteria acquire and incorporate DNA segments from other bacteria that have released their DNA complement into the environment after cell lysis, can move resistance genes into previously susceptible strains (Tenover, 2006).

Mutation and selection, together with the mechanisms of genetic exchange, enable many bacterial species to adapt quickly to the introduction of antibacterial agents

into their environment. Although a single mutation in a key bacterial gene may only slightly reduce the susceptibility of the host bacteria to that antibacterial agent, it may be just enough to allow its initial survival until it acquires additional mutations or additional genetic information resulting in fullfledged resistance to the antibacterial agent (Tenover, 2006).

2.5. PROFILE OF MICROBES USED FOR THIS STUDY

2.5.1. Klebsiella pneumoniae

Klebsiella pneumoniae is among the most common Gram-negative bacteria encountered by physicians worldwide (Wen-Chien *et al*., 2002). It is a common hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumonia, and intra-abdominal infections. *K. pneumoniae* is also a potential community-acquired pathogen (Wen-Chien *et al*., 2002). It is an encapsulated, facultatively anaerobic bacterium and can also overcome innate host immunity through several means (Wen-Chien *et al*., 2002). The polysaccharide capsule, which is the main determinant of their pathogenicity is composed of complex acidic polysaccharides and its massive layer protects the bacterium from phagocytosis by polymorphonuclear granulocytes. In addition, the capsule prevents bacterial death caused by bactericidal serum factors (Wen-Chien *et al*., 2002). This is accomplished mainly by inhibiting the activation or uptake of complement components, especially C3b. The bacteria cells also produce multiple adhesins which may be fimbrial or nonfimbrial, each with distinct receptor specificity and with these the microorganism is able to adhere to the host cells, which is critical to the infectious process **(**Umeh and Berkowitz, 2009**).** According to O'Brien *et al*., (1985) the antibiotic resistance

factor of *K. pneumoniae* isolates from 12 medical centers worldwide, over a one to six year period, showed resistance to ampicillin and carbenicillin. Resistance to other antibiotics was less frequent with isolates of *K. pneumoniae* from 5 of 6 United State (US) centers than with those from 6 centers outside the US (O'Brien *et al*., 1985). In nearly all of these centers, resistance to sulfamethoxazole-trimethoprim, gentamicin, tobramycin, or chloramphenicol was more frequent in isolates of *K. pneumoniae* than in those of *Escherichia coli*, while the reverse was true for resistance to tetracycline (O'Brien *et al*., 1985). Resistance to multiple antibiotics declined gradually in isolates of *K. pneumoniae* at one centre, but rose abruptly again with dissemination of a new plasmid (O'Brien *et al*., 1985).

2.5.2. Staphylococcus aureus

Staphylococcus aureus causes boils, styes, pustules, impetigo, infections of wounds (cross infections), ulcers and burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia, and pleular empyema. Also, included are toxic food-poisoning (rapid onset on fever), toxic shock syndrome and toxic skin exfoliation (Lowy, 2003). According to Cheesbrough (2000), *S. aureus* is carried in the nose of 40% or more of healthy people. It is a pathogen of great concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections, and its capacity to adapt to different environmental conditions (Lowy, 2003). The mortality of *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials (Lowy, 2003) and it is now the leading overall cause of nosocomial infections.

2.5.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic human pathogen associated with nosocomial infections of immunocompromised individuals as a result of burns or other severe trauma, underlying diseases, including cancer, diabetes and cystic fibrosis (CF), deliberate immunosuppression and major surgery (Poole, 2001). *P. aeruginosa* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections. Infections are often difficult to eradicate due to *P. aeruginosa* being resistant to many antimicrobials (Cheesbrough, 2000) and this intrinsic resistance has long been attributed to the outer membrane, a barrier of limited permeability (Poole, 2001). A study on the resistance of *P. aeruginosa* isolates from pus samples obtained from wound of patients in Enugu and Abakaliki using the paper disc diffusion technique showed that, out of fifty pus samples screened, 34(64%) yielded *P. aeruginosa* (Amadi *et al*., 2009). The sensitivity of the *P. aeruginosa* was then investigated using amoxycillin, co-trimoxazole, streptomycin, gentamicin, chloramphenicol and ciprofloxacin and the highest resistance obtained was recorded for amoxycillin (88.2%), followed by co-trimoxazole (76.5%), streptomycin (67.6%), gentamicin (58.8%), chloramphenicol (58.8%) and ciprofloxacin (23.5%) (Amadi *et al*., 2009).

2.5.4. Staphylococcus saprophyticus

Staphylococcus *saprophyticus* is most commonly identified in urinary tract infections (UTIs) especially in sexually active young women (Cheesbrough, 2000). Individuals colonized with this organism can be treated with antibiotics and then usually make a full recovery unless they have compromised immune systems or unusual

complications (Cheesbrough, 2000). It is a coagulase negative *Staphylococcus*, and the second most common cause of acute cystitis after *E. coli* in young females, causing 5 to 25% of all cystitis (Nicollet and Harding, 1982). According to a study by Gupta *et al*., (1999) *E. coli* and *S. saprophyticus* were the most common uropathogens, accounting for 90% of the 4342 urine isolates studied.

2.5.5. *Proteus mirabilis*

Proteus mirabilis is widely distributed in water and soil in the natural environment (Cheesbrough, 2000). As part of the normal bacteria that is found in the intestines, *P. mirabilis* aids in the digestion process. However, when it is spread into the urinary system, it can cause infection especially in the elderly (Cheesbrough, 2000). Most infections with *P. mirabilis* are associated with the presence of renal stones (Cheesbrough, 2000). The organism is often a secondary invader of ulcers, pressure sores, burns and damaged tissues. It also causes septicaemia and occasionally meningitis and chest infections (Cheesbrough, 2000). According to Mahamat *et al*., (2006) one thousand and eight (1008) non-duplicate urinary tract isolates from 1999 to 2005 at Nîmes University Hospital studied showed 63.1% from females with a mean age of 76 years and a mean resistance rate of 59.0% for amoxicillin (AMX), 48.0% for piperacillin (PIP), 3.9% for cefotaxim, 33.9% for amoxicillin plus clavulanic acid (AMC) and 2.8% for piperacillin plus tazobactam (TZP).

2.5.6. Salmonella typhi

Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella typhi* that lives only in humans. Typhoid fever remains a serious health threat especially in

developing countries where it is estimated that over 20 million cases occur annually resulting in greater than 700,000 deaths globally (Thong *et al*., 1994). Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. A small number of persons who recover from typhoid fever remain carriers of the bacteria. In both cases, *S. typhi* are shed in the faeces (Cheesbrough, 2000). It has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos **(**Brusch *et al*., 2010)**.** The antibiotic of choice for many years was chloramphenicol, but like many pathogens, chloramphenicol-resistant strains have emerged. In addition, many strains have developed resistance to ampicillin and trimethoprim/sulfamethoxazole, which are considered appropriate alternatives to chloramphenicol (Mills-Robertson *et al*., 2002). In a study by Mills-Robertson *et al*., (2003), 30 strains of bacteria out of a total of 58 isolates (52%) exhibited multiple drug resistance (MDR) with 10 strains being resistant to all three 'first line' antibiotics.

2.5.7. Escherichia coli

Escherichia coli is one of the main causes of nosocomial infections in humans (Olowe *et al*., 2008). It is naturally found in the intestinal tract, in soil and water. *E. coli* causes infections of wounds, peritonitis, sepsis, endotoxin induced shock, diarrhoeal disease, meningitis and bacteraemia in neonates and it is also the commonest pathogen isolated from patients with cystitis with recurring infections being common in women (Cheesbrough, 2000). The organism is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections (Olowe *et al*., 2003). It is one of the organisms most

frequently isolated from different clinical cases of diarrhoea and others (Okeke *et al*., 1999; Olowe *et al*., 2003). A study by Olowe *et al*., (2008) demonstrated *E. coli* multi-drug resistance in isolates from clinical samples obtained from patients at Ladoke Akintola University Teaching Hospital, Osogbo, Osun State, Nigeria. Seven antimicrobials were used during the study and the prevalence of strains resistant to antimicrobials were; Tetracycline (91.6%), Ampicillin (86.7%), Sulphnamide (77.8%) and Gentamicin and Nalidixic acid which were (39.3%) and (4.1%) respectively. A total of seven antibiotic resistance profiles were obtained with over 64% of the isolates showing multi-drug resistance. The isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range less than 6–25 kb. Very large resistance levels greater than 85% were detected against Tetracycline, Sulphnamide, and Cotrimoxazole while Nalidixic acid showed least resistance of 4.1% among the isolates (Olowe *et al*., 2008). Majority of the isolates were positive for betalactamase production when subjected to starch paper method (Olowe *et al*., 2008).

2.5.8. Salmonella typhimurium

Until recently the most common cause of food poisoning by *Salmonella* species was due to *Salmonella typhimurium* (Gallardo *et al*., 2003). The disease is characterized by diarrhoea, abdominal cramps, vomiting and nausea, and generally lasts up to 7 days (Cheesbrough, 2000). In the elderly, young, or people with depressed immune systems, *Salmonella* infections are often fatal if they are not treated with antibiotics (Cheesbrough, 2000). Antibiotic resistance in clinical isolates of *S. typhimurium* has steadily risen in recent years (Gallardo *et al*., 2003). For instance, resistance to some

β-lactam antibiotics, tetracyclines, chloramphenicol, or trimethoprim is reported with increasing frequency, although quinolones are an alternative therapy, however, the development of quinolone resistance in *Salmonella* spp. during therapy of patients with enterocolitis has been reported (Ruiz *et al*., 1999). In a study conducted in India to investigate antibiotic resistance and epidemiological relationships for five multiresistant strains of *S. typhimurium* isolated from fish, four strains showed resistance to nalidixic acid, chloramphenicol, tetracycline, co-trimoxazole, gentamicin and β -lactam antibiotics (Ruiz *et al*., 1999). The remaining strain was susceptible to all β -lactam antibiotics tested and to co-trimoxazole but resistant to the other antibiotics tested (Ruiz *et al*., 1999).

2.6. ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF EXTRACTS

Antimicrobial properties of plants have been investigated by a number of researchers worldwide (Nascimento *et al*., 2000). For instance, in Argentina, a research tested 122 known plant species used for therapeutic treatments (Anesini and Perez, 1993) and found out that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger* with extract from *Tabebuia impetiginosa* among the plants reported to have the most potent compound (Anesini and Perez, 1993).

Another study has revealed that, substances extracted from nine known plants in Uruguay did not show any activity against *C. albicans* and *Saccharomyces cerevisiae*, but inhibited the growth of *Bacillus subtilis, E. coli*, and *P. aeruginosa* (Alonso-Paz *et al*., 1995). Nwinyi *et al.*, (2009) also screened the aqueous and

ethanol extracts of *Ocimum gratissimum* and *Piper guineense* leaves for antibacterial activity against *E. coli* and *S. aureus* and found that both extracts exhibited selective inhibition against the isolates with the Minimum Inhibitory Concentration (MIC) determined by the agar diffusion method ranging between 2.50 and 10.00mg/ml. Their ethanol extracts showed higher inhibitory effect compared to the aqueous extracts (Nwinyi *et al.,* 2009).

Several other studies have been conducted in Ghana to demonstrate the antimicrobial properties of medicinal plants and their potential as an alternate for the treatment of infectious diseases (Agyare *et al*., 2006; Dayie *et al*., 2008; **Pesewu *et al.*, 2008)**. In one of such studies, the methanol and petroleum ether extracts of the leaf and stem bark of *Nauclea latifolia, Bridelia atroviridis* and *Zanthoxylum gilletii* showed antimicrobial activity against the test organisms (Agyare *et al*., 2006). Results from their study supported some of the folkloric uses of these plants as agents for the management of sores, gonorrhea, dysentery, wounds and toothache (Agyare *et al*., 2006).

Dayie *et al*., (2008) also investigated group of solvents (petroleum ether, dichloromethane, methanol, and water extracts) most suitable to extract the active constituents from *Ageratum conyzoides* and subsequently screened the crude extracts for antimicrobial activities against typed cultures of *S. aureus NCTC 6571*, *Methicillin Resistant S. aureus NCTC 12493* and clinical isolates of resistant strains of *S. aureus, P. aeruginosa,* and *E. coli* (Dayie *et al*., 2008). It was found that, the methanolic extract inhibited the growth of all the strains of *S. aureus* with a zone size ranging from 26-28 mm in diameter (Dayie *et al*., 2008). However, it was weakly

active against *E. coli* and had no inhibitory activity against *P. aeruginosa*. Water extracts and 40-60% Petroleum ether extracts, did not show any antibacterial activity against all the test microorganisms (Dayie *et al*., 2008). They concluded that, 98% methanol was the best solvent among the three (Dayie *et al*., 2008).

In an ethno botanical survey carried out in the Akuapem-North district of the Republic of Ghana**,** Pesewu *et al*., (2008) investigated the antimicrobial activity of Chloroform, ethanol and aqueous extracts of traditional Ghanaian medicines with special interest in anti-methicillin-resistant *S. aureus* (MRSA) activity. Extracts of 13 plant species inhibited the growth of one or more of the following bacteria: MRSA, methicillin-sensitive *S. aureus* (MSSA), *Streptococcus pyogenes*, *E. coli*, *P. aeruginosa,* and *Proteus vulgaris* while extracts from 11 of these 13 plant species also inhibited the growth of three or more of 14 additional clinical isolates of MRSA. Aqueous extracts of *Alchornea cordifolia* were active against all 21 bacterial strains tested and showed the highest levels of antibacterial activity overall with MIC's against MRSA in the range of 1.6–3.1 mg/ml and MBC's in the range of 6.3–12.5 mg/ml. In the study, the presence of antibacterial activity in extracts of *Elaeophorbia drupifera*, *Rauwolfia vomitoria* and the leaves of *Solanum verbascifolium*, plants traditionally used to treat skin infections, was reported for the first time while extracts from *Alchornea cordifolia,* also used to treat wounds, had the widest spectrum of antibacterial activity (Pesewu *et al*., 2008**)**. Thus, medicinal plants with antimicrobial properties exist and all efforts must be exhibited to explore them. Consequently, it is in the spirit of further exploration that the following plants were selected for this study.

Plants are a rich reservoir of antimicrobials with a single plant containing several active principles of biological significance (Kumaraswamy *et al*., 2008). For instance, many pharmacological activities have been reported about saponins (Soetan

et al., 2006), phenolics (San Francisco and Cooper-Driver, 1984; Onwukaeme *et al*., 2007), and flavonoids (Rattanachaikunsopon and Phumkhachorn, 2010) as being antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer. An alkaloid, cryptolepine is also known to cause cell lyses and morphological changes in *S aureus* (Sawer *et al*., 2005) while tannins are known to possess antimicrobial properties with the ability to bind to proteins of exposed tissues, thus precipitating the protein. Thus, phytochemicals aid animals and man by creating a preventive barrier against diseases and sicknesses (Abba *et al.,* 2009).

2.6.1. *Phyllanthus fraternus*

The plant *Phyllanthus fraternus* Webster (Family**:** *Euphorbiaceae)* commonly called; gulf leaf- flower is a small, erect, annual herb (Dicotyledonous) that grows 30-40 cm in height (Wunderlin and Hansen, 2002). It is an herbaceous weed commonly found on roadsides, cultivated land, waste forest areas and savanna, and generally rare in Senegal, Ivory Coast, Ghana and Nigeria (Burkill, 1985). It is widely distributed in Asia and in the West Indies and probably native to Western India and Pakistan (Burkill, 1985). The whole plant is very bitter due to the presence of the alkaloid phyllanthin (Burkill, 1985). Alkaloids hypophyllanthin and quercetin are also present amongst a number of active substances as well as saponin (Burkill, 1985). Notwithstanding the bitter taste, all livestock are said to browse it in Senegal, at Lake Chad (Adam, 1966), in Kordofan and elsewhere (Burkill, 1985). It has been cited as a fodder for horses in Java (Burkill, 1985). The plant-extract was officially used at one time in a Government hospital in Accra to allay griping in dysentery, and other painful spasmodic affections of the intestines (Burkill, 1985) where the

administration of opium and morphia was not advisable. The leaves are rich in potassium by which they possess a strongly diuretic property (Burkill, 1985). The pounded leaves are taken in Ghana against gonorrhoea and (unspecified) parts of the plant are used against constipation (Burkill, 1985). In Ivory Coast it is used in cases of difficult childbirth, costal and fever pains, sore-throat and oedemas (Burkill, 1985).

In India, Malaysia and the Philippines it has a miscellaneous usage for gastro-intestinal symptoms, dropsy and urino-genital diseases (Taylor, 2005). The fresh roots are said to be beneficial against jaundice and are taken with milk as a galactogogue (Taylor, 2005). Poultices are applied to caterpillar urticarias, skin-complaints, oedematous swellings and ulcers while the latex is applied to sores and ulcers, and mixed with oil, for ophthalmias. The young leaves are used against cough and hiccups in children and also in India for mild forms of intermittent fever (Taylor, 2005). The plant is also employed for the treatment of blennorrhagia, colic, diabetes, dysentery, fever, flu, tumors, jaundice, vaginitis, and dyspepsia (Taylor, 2005).

P. fraternus is a medicinal plant that has undergone extensive phytochemical research spanning over four decades. Geraniim, an alkaloid, contained in this plant is seven times more potent as analgesic than aspirin or acetaminophen (Khanna *et al*., 2002). The aqueous extracts have also been shown to increase the lifespan of mice with liver cancer (RajeshKumar *et al*., 2000) and also exhibit HIV-1 reverse transcriptase inhibition activity (Ogata *et al*., 1992).

2.6.2. *Cymbopogon citratus*

Cymbopogon citratus (Family**:** Poaceae) is a tall, aromatic, coarse grass of 1.5 m high. It is a monocotyledonous hypogeal perennial plant with slender sharp edged green leaves that has a pointed apex (Asaolu, 2009). The stem is reddish brown in colour and it is attached to the bulb by stalk. The entire plant is attached to the soil by fibrous root (Asaolu, 2009). *C. citratus* is known as Guatemala, West Indian, or Madagascar lemongrass. *C. citratus* is cultivated in the West Indies, Central and South America, and most tropical regions (National Plant Data Center, 2008). Freshly cut and partially dried leaves are used medicinally and are the source of the essential oil (National Plant Data Center, 2008).

Many biologically active substances have been isolated and elucidated in *C. citratus* (Asaolu, 2009). The most important being citral, which aids digestion as well as relieve spasms, muscle cramps, rheumatism and headache (Asaolu, 2009). A tea made from the leave of *C. citratus* has been used to treat fever, cold, cough and stomach upset. The tea also has diuretic properties and can reduce urinary and water retention difficulty (Asaolu, 2009). Extracts of both the leaves and stalks of *C. citratus* are used as an herbal medicine to treat nervous condition and inflammation (Asaolu, 2009).

Many reports describe the antimicrobial effects of lemongrass, including activity against both Gram-positive and Gram-negative bacteria and fungi (Onawunmi *et al*., 1984; Ogunlana *et al.*, 1987; Kishore *et al*., 1993; Lima *et al*., 1993; Mishra and Dubey, 1994; Wannissorn *et al*., 1996; Qureshi *et al*., 1997; Hammer *et al*., 1999; Helal *et al*., 2006). The effects are attributed in part to the geraniol (alpha-citral) and

neral (beta-citral) constituents (Onawunmi *et al*., 1984). Clinical trials are lacking, however in a 13-oil study, lemongrass oil was found to be among the most active against human dermatophyte strains, inhibiting 80% of strains, with inhibition zones more than 10 mm in diameter (Lima *et al*., 1993).

2.6.3. *Psidium guajava*

Psidium guajava Linn. (Family: Myrtaceae), guava, grows as a large spreading shrub or a small tree up to 15 m high (Jaiarj *et al*., 1999). Guava is an evergreen growing wild plant in torrid zones and subtropics with very thin skins. Its leaves are evergreen, opposite, short petioled, oval or oblong (Akinpelu and Onakoya, 2006). The leaves contain an essential oil rich in cineol, tannins, triterpenes and flavonoids (Olajide *et al*., 1999) as well as guajava polyphenol which has an anti-oxidation action while the flower and leaf of the plant have been reported to have antimicrobial activity (Nair and Chanda, 2007). Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments such as wounds, ulcers, bowels and cholera (Sanches *et al*., 2005). The plant is also used in folk medicine to treat fevers, diarrhoea, and as a tonic in psychiatry cases (Oliver-Bever, 1986). The boiled water extract of guava plant leaves and bark are used as remedies for dysentery, diarrhoea and upper respiratory tract infections (Nair and Chanda, 2007). In Malaysia, *P. guajava* is used for stomach ache and gastroenteritis (Nair and Chanda, 2007). Also the leaf, root, and bark extracts are used for the treatment of diarrhoea, leukorrhea, cholera, external ulcers, and skin diseases (Nair and Chanda, 2007).

According to many Researchers including, Jaiarj *et al*., (1999); Mills-Robertson, (2004); Qa'Dan *et al*., (2005), *P. guajava* extract is known to have varying degree of antimicrobial activity which is attributed to the presence of tannins, triterpenoids and flavonoid glycosides in the leaves (Qa'Dan *et al*., 2005). A strong antimicrobial action of guava leaves on Gram-positive and Gram-negative organisms has also been reported by Oliver-Bever, (1986) and Mills-Robertson, (2004) whilst the extract *in vitro* has been shown to have antimicrobial activity against *E. coli, S. typhi, S. aureus, P. mirabilis,* and *S. dysenteria* (Iwu, 1993).

2.6.4 *Hoslundia opposita*

Hoslundia opposita Vahl (**Family:** Lamiaceae) is an herbaceous perennial shrub and sometimes soft, which grows up to 1.2 m high. Common names in English include orange bird berry and bird gooseberry (Mukoma, 2005). It is widely distributed in tropical and subtropical open lands of Africa (Mujovo, 2009). *H opposita* is used in ethnomedicine to treat sore throats, colds, sores, veneral diseases, herpes and other skin diseases, malaria, microbial infections, epilepsy, fever and inflammation (Annan and Dickson 2008). Infusion of its leaves is widely used in traditional medicine as a purgative, diuretic, febrifuge, antibiotic and antiseptic (Mujovo, 2009). The crude extract of the entire plant have been found to exhibit strong antibacterial activity (Mujovo, 2009). A recent study has reported that leaves of this plant could be potentially used in the treatment of epilepsy and convulsions (http://www.plantzafrica.com/planthij/hoslundop.htm).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Study Site and Clinical Specimen

The pathogenic microorganisms used including *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* were isolated at the Microbiology laboratory at the Komfo Anokye Teaching Hospital (KATH), Kumasi. Isolation and identification of the microorganisms from blood, urine and wound swab were performed at KATH while evaluation of the antimicrobial properties of the selected medicinal plants was done in the laboratories of the Microbiology Department, Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem.

3.1.2. Chemicals, media, and reagents

Iodonitrotetrazolium chloride (INT) (Fluka Biochemika Sigma-Aldrich, Austria). Nutrient agar (Merck, Germany). Bacteriological Peptone, and Dimethylsulphoxide (DMSO) were obtained from Sigma-Aldrich, Germany. Mueller-Hinton agar, Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 iu/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc) were purchased at Lynch Medical Service, Accra-Ghana.

3.1.3. Equipment

Equipment used include Binder hot air oven, Webeco-Bad Scwartau autoclave, Eyelashaker water bath from Rikakikai Company limited Tokyo, Binder incubators and Telstar Laminae flow cabinet, Heidolph rotary evaporator (LABOROTIA 4000, Germany), and Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 gr., Germany)

3.1.4. Medicinal plant material

All the medicinal plants used namely *Phyllanthus fraternus, Hoslundia opposita, Psidium guajava* and *Cymbopongon citrates* were collected and identified by a Taxonomist from the Plant Development Department (PDD) of CSRPM and voucher specimens of each plant kept at the CSRPM herbarium.

3.2. METHODOLOGY

3.2.1 SAMPLE COLLECTION AND ISOLATION OF MICROORGANISMS

Clinical samples were collected using sterile swabs or containers depending on the specimen being collected which include blood, urine and wound swab then cultured into a suitable sterile medium in order to maintain appropriate quality control of laboratory findings.

3.2.2 IDENTIFICATION OF MICROORGANISMS

3.2.2.1 Gram Stain

A Gram stain and culture of the clinical isolates are the most commonly performed microbiology tests used to identification of microorganisms. One drop of saline was placed on a slide. Colonies from the agar plates were picked using a sterile loop, mixed with saline and smeared over the surface of the slide. The smears were allowed to dry thoroughly and fixed by passing the slide, smear up, quickly through the Bunsen flame three times. The smears were allowed to cool before staining. The reagents Crystal violet, Gram's iodine, Ethanol (decolouriser), Carbol fuchsine was added for 60 seconds each. Between each staining reagent the smear was washed under a gently running tap, excess of water tipped off before the next reagent was added (Chessbrough, 2000).

3.2.2.2 Microscopy (morphology)

The Gram stained smears were examined microscopically using the 40X objective lenses to check to staining and distribution of material, and then with the oil immersion objective to report the bacteria (Chessbrough, 2000).

3.2.3 Staphylococcus aureus and Staphylococcus saprophyticus

Isolates were incubated at 37°C for 24 hours on blood agar. Growth with morphological characteristics suggestive of *Staphylococci* was tested using preliminary test such as Gram stain, catalase test and tube Coagulase. Coagulase test aids in differentiating *S. aureus* from *S. saprophyticus* while catalase test differentiates *S. saprophyticus* from *S. epidermidis* (Chessbrough, 2000).

3.2.3.1 Catalase test

This test is commonly used to differentiate catalase producing bacteria (*S. epidermidis*) from non-catalase producing bacteria (*S. saprophyticus*). A test tube was filled with 2-3 ml of 3% Hydrogen peroxide (H_2O_2). A distinct colony was picked using a sterile wooden stick or a glass rod and immersed in the test tube containing H_2O_2. Evolution of gas indicated catalase activity (Chessbrough, 2000).

3.2.3.2 Tube Coagulase

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in a test tube. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci. Three test tubes were labeled "test", "negative control" and "positive control". Each tube was filled with 0.2 ml of human plasma using a pipette. To the tube labeled test, 0.8 ml of overnight broth culture of test bacteria was added. To the tube labeled positive control, 0.8 ml of overnight broth culture of known *S. aureus* was added and to the tube labeled negative control, 0.8 ml of sterile broth is added. All the tubes were incubated at 37^{o}C and observed for up to four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube. If the test remains negative until four hours at 37^{o}C, the tube is kept at room temperature for overnight incubation (Chessbrough, 2000).

3.2.4 Klebsiella pneumoniae Salmonella typhi, Salmonella typhimurium, Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli

Enterobacteriaceae from various clinical sources were cultured on blood and MacConkey agar at 37°C for 24 hours. The top portion of a single well isolated colony was inoculated into sets of media and substrates including Triple Sugar Iron (TSI) agar, Urea agar and Citrate agar for identification (Chessbrough, 2000).

3.2.4.1 PRESUMPTIVE IDENTIFICATION

Agar plates were examined to determine growth characteristics and colony appearance of the isolates. The morphology of the pure colonies was determined using Gram staining technique. Initial test including oxidase test and indole test were also demonstrated.

3.2.4.1.1 Oxidase test

Oxidase test was used to assist in the identification of *Pseudomonas* which produce the enzyme cytochrome oxidase. 2 to 3 drops of freshly prepared oxidase reagent was added to a piece of filter paper in a clean petri dish. A colony of the test organism was smeared on the filter paper using a piece of stick or glass rod. The development of a blue-purple colour within a few seconds indicates the presence of the enzyme cytochrome oxidase and a positive oxidase test (Chessbrough, 2000).

3.2.4.1.2 Sulphur Indole Mobility (SIM) agar test

Most strains of *E. coli* break down tryptophan with the release of indole. In this test, the test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and incubated at 37°C for up to 48 hours. 0.5 ml of Kovac's reagent was added

to the test, mixed gently and examined for a red colour in the surface layer within 10 minutes. Also a drop of peptone water culture of the test microbe was placed on a clean slide and covered with a cover slip making sure that no air bubbles were trapped. The film was then viewed under the microscope with the low power objective lens, with the condenser sufficiently closed to allow contrast and then later viewed with the high power objective lens. Care was taken to observe true locomotion but not passive drifting and Brownian motion. The SIM agar was also used to confirm the mobility of the microorganism. A straight inoculation rod was sterilized by flaming. The top of a well isolated colony was touched and then inserted straight into the agar and the inoculated agar was incubated at 37 ºC overnight. Motile microorganisms were found to diffuse laterally into the agar along the line where the straight rod was inserted. No such diffusion in the agar was observed for non-motile microorganisms (Chessbrough, 2000).

3.2.4.2 CONFIRMATION OF IDENTIFICATION

Biochemical tests were used for the identification of the isolated microbes. These included Urea agar test, Sulphur Indole Motility agar (SIM) test and Triple Sugar Iron (TSI) agar test.

3.2.4.2.1 Simmons citrate agar test

Simmons citrate agar, a synthetic medium is recommended for the differentiation of Enterobacteriaceae, based on whether or not citrate is utilised as the sole source of carbon. Citrate agar slants were each inoculated with a loopful of the test microorganism and incubated overnight at 37 ºC. Positive growth produced an

alkaline reaction and changed the colour of the medium from green to bright blue, whilst in a negative test the colour of the medium remained unchanged (Chessbrough, 2000).

3.2.4.2.2 Urea agar test

Urea agar test is used for the differentiation of *Enterobacteriaceae* based on their ability to hydrolyse urea. Some species of *Proteus* for example may give similar reactions to *Shigellae* and *Salmonellae* after Triple Sugar Iron (TSI) agar test, for this reason TSI agar is used alongside Urea agar test. Briefly, colonies were picked from using a straight inoculating wire and inoculated into separated urea agar slopes. The urea agar was incubated at 37 ºC for 5 hours. Urease positive microbes turned the inoculated slopes pink whereas in the case of the urease negative microbes, the urea agar plates remained colourless or yellow (Chessbrough, 2000).

3.2.4.2.3 Triple Sugar Iron (TSI) agar test

TSI is a composite medium for the differentiation of Enterobacteriaceae according to their ability to ferment lactose, sucrose and glucose, and to produce hydrogen sulphide (Chessbrough, 2000). It contains phenol red which is a pH indicator. Below pH 6.8 it turns yellow and above 8.2 it turns red. Not only does this medium perform most of the functions of Kligler Iron Agar but, in addition, its sucrose content permits the recognition and exclusion of sucrose-fermenting species (Chessbrough, 2000). A straight inoculation rod was sterilized by flaming. The top of a well isolated colony was touched and used to streak the slope, with the butt stabbed and incubated at 37 ºC overnight. Failure to turn the butt yellow indicates that no fermentation has occurred, and that the bacterium is an obligate aerobe. Acid

production limited to only the butt of the tube is an indicative of glucose utilization. This is because the concentration of glucose is lower than that of other sugars, thus the acid production is not very extensive. A large amount of acid turned both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose. A few bacteria were capable of reducing the SO_4 to H_2S (hydrogen sulfide). The iron combines with the H_2S to form FeS (ferrous sulfide) a black compound and this turned the butt black. Thus, a black butt indicated H_2S production (Chessbrough, 2000).

3.2.5. Antibiotics sensitivity test

The *in vitro* antibiotic sensitivity test was performed using Kirby-Bauer disc diffusion method. Two to six hours cultures of the microbes in peptone water that had achieved the 0.5 McFarland standard turbidity were flooded over Muller-Hinton agar and antibiotic disc were carefully placed on the surface of the agar, allowed to dry, then placed in the incubator at 37 ºC for 16-18 hours. The zones of inhibition of the various antibiotics were measured with a meter rule by taking the diameter of the zones. They were compared with the standard antimicrobial sensitivity chart and recorded as sensitive or resistant to the antibiotics. The antibiotics that were tested included; Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 i·u/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), and some of the newer generation antibiotics including Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc). The antibiotic susceptibility tests on the isolates were done according to the guideline set by the Clinical and Laboratory Standards Institute (CLIS, 1993).

3.2.6. Herbal preparations

3.2.6.1. Preparation of Ethanolic Extract

Five hundred grams (500 g) of each pulverized plant materials was cold macerated with 70% ethanol for three days. The ethanolic extracts were concentrated using Heidolph rotary evaporator (LABOROTIA 4000, Germany) at a temperature of 65 ^{o}C. Twenty five millilitres (25 ml) portions of the concentrated ethanolic extracts were poured into flasks and lyophilized using a Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 gr., Germany) for 24 hours. The dried powders were stored in air-tight containers and refrigerated until needed.

3.2.6.2. Preparation of Aqueous Extract

Aqueous fractions (decoctions) of the plant materials were prepared by boiling 1000 g of the dried plant material in 10 Liters water for between 30-45 minutes. The resultant extracts were concentrated using reduced temperature for another 60 minutes. The resultant extracts were lyophilized as described above and stored in a refrigerator until needed.

3.2.7. Preparation of stock solutions for the bioassays

3.2.7.1. Herbal extracts

In the preparation of stock solutions for the bioassays, 3.2 g/10 ml of the lyophilized ethanol products were reconstituted using 20% DMSO for the determination of the antimicrobial activity assay. The stock solutions were stored in a refrigerator until needed. Quantities of 3.2 g of lyophilized aqueous products were reconstituted in 10

ml of sterile distilled water for the antimicrobial activity determination. The stock solutions were stored in a refrigerator until needed.

3.2.7.2. Culture media

Nutrient Agar, Bacteriological Peptone, and Mueller-Hinton agar were prepared according to manufacturer's instructions. The stock cultures of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus,* and *Staphylococcus saprophyticus* were sub-cultured onto fresh Nutrient agar plates, incubated for 24 hours and stored in a refrigerator until needed. When needed, three to five well-isolated colonies of the same morphological type of the microbes were suspended in test tubes containing 5 ml of sterilized Bacteriological Peptone and incubated for 16 hours and then sub-cultured at 37ºC for another 2 hours to attain the turbidity of 0.5 McFarland standard. The turbidity of the actively growing broth cultures was adjusted with sterile Bacteriological Peptone to obtain turbidity optically comparable to that of 0.5 McFarland Standard (approximately 1-2 x 10^8 CFU/ml for *E. coli* ATCC 25922) as recommended by the National Center for Infectious Disease, Center for Disease Control and Prevention (1999).

3.2.8. Phytochemical analysis

The phytochemical screening of all the extracts was carried out to determine the presence of the following compounds; alkaloid, flavonoids, polyuronides, reducing sugars, cyanogenic glycoside, saponins, terpenes, anthracenosides, phytosterols and phenols as described below (Sofowora 1993).

3.2.8.1. Saponins (the Froth test)

2 ml of the extract was added to distilled water and shaken vigorously. A froth (foam) that persisted for more than 10 minutes indicated the presence of saponins (Sofowora 1993).

3.2.8.2. Cyanogenic glycosides

The presence of hydrocyanic acid (HCN) is detected based on the release of HCN on hydrolysis of glycosides. Five milliliters of distilled water was added to an aliquot of the extract and a piece of Sodium Picrate paper was suspended above the extract by tapping the top edge between the cork and the tube wall. This set up was allowed to stand for 30mins. The colour change of the Sodium picrate paper from yellow to brick red due to the formation of Sodium isopurpurate indicated the release of HCN (Sofowora 1993).

3.2.8.3. Polyuronides / Polyamides

Ten milliliters of acetone was added to 2ml of the extract in a test tube. The appearance of a precipitate indicated the presence of polyuronides (Sofowora 1993).

3.2.8.4. Reducing sugars

Two milliliters of the extract was diluted in 2ml of distilled water and Fehling's solutions (A+B) added to the mixture. A brick red precipitate after standing in the heat or water bath indicated the presence of reducing sugars (Sofowora 1993).

3.2.8.5. Alkaloids

Twenty milliliters of the alcohol extract was evaporated to dryness on a water bath. Five to ten milliliters of 10% hydrochloric acid (HCl) and $CHCl_3$ were added to the extract. Concentrated ammonia was added to the aqueous layer to obtain a pH of between 8 and 9. The solution was then extracted in a separating tube with chloroform or ether. The apolar solvent was evaporated to dryness in an evaporated dish in a water bath and the residue was dissolved with 5ml of HCl (2N) and the solution was divided into three separate test tubes. Two to three drops of Mayer's reagent was added to one and the same amount of Bertrand's reagent to the other, while the third test tube served as a reference. The appearance of an opalescent or yellow-white precipitate with the reagents indicated the presence of alkaloids (Sofowora 1993).

3.2.8.6. Anthracenocides

Four milliliters of the ether extract was concentrated to 2ml with 2ml of 25% of ammonia solution added and shaken. A cherry red colour of the alkaline layer indicated the presence of emodols (aglycones of anthracenosides) in an oxidized form–Borntrager's reaction (Sofowora 1993).

3.2.8.7. Flavonosides

Five milliliters of the ether extract was evaporated to dryness. The residue was dissolved in 2ml of 50% methanol by heating and 4 grams of metal magnesium and 6 drops of concentrated HCl added. A red solution indicated the presence of flavonoids, while an orange solution indicated the presence of flavones - Shibata's reaction (Sofowora 1993).

3.2.8.8. Phenolic substances

Two to three drops of 10% Ferric chloride solution was added to 5ml of extract in a test tube and observed. Blue black precipitate indicated the presence of gallic tannins while dirty green precipitate indicated the presence of catachol tannins (Sofowora 1993).

3.2.8.9. Sterols and Triterpenes

Ten milliliters of the ether extraction was evaporated to dryness. The residue was dissolved in 0.5ml of acetic aldehyde and 0.5ml of $CHcl_3$ added and transferred into a dry test tube. About two milliliters of concentrated sulphuric acid (H_2SO_4) was added to the bottom of the tube using a pipette. A brownish red or violet ring at the contact zone of the two liquids indicated the presence of sterols and triterpenes. The greenish and brownish red (wine) nature of the supernatant indicated the presence of sterols and triterpenes respectively (Sofowora 1993).

3.2.9. Determination of the potency of the herbal preparation

The agar diffusion method was used to investigate the antimicrobial properties of the crude extracts as described in the Clinical and Laboratory Standards Institute (2000) and the National Center for Infectious Disease, Center for Disease Control and Prevention (1999). Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterilized swab was aseptically dipped into the suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid to remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface with bacteria.

This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. A sterilized cock borer of an internal diameter of about 6 mm was used to punch holes in the medium and 32% w/v of plant extracts were dispensed into the respective labeled holes. Discs of Standard drug 30 µg/disc chloramphenicol was used as positive controls while 20 % v/v DMSO was used as negative controls. Triplicates of each plate were made and the procedure was repeated for the other microorganisms. The plates were kept in the refrigerator for about 4 hours for complete diffusion of the extract and incubated at 37 ºC for 48 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a sterilized ruler Clinical and Laboratory Standards Institute (CLIS, 2000).

3.2.10 Determination of MICs and MBCs of the crude extracts

The MIC values of the crude extracts were determined using the microplate dilution method as described by Eloff (1998). One Hundred microliters of 32 mg/ml of the ethanol extract was added to 100 µl of sterile Bacteriological Peptone in the first well in the 96-well microplate and mixed well with a micropipette. Hundred microliters of this dilution was added to Bacteriological Peptone in the next well in the column and the process repeated to yield a two-fold serial dilution in the original extract. The process was repeated for the other plant extracts in other columns of the microplate. A reference solution of chloramphenicol was also serially diluted in another column of the microplate as a positive control test. Hundred microliters of an actively growing test organism was added to each of the dilutions and the microplate covered. A triplicate of each microplate was made and the procedure was

repeated for the other organisms. The microplates were incubated at 37 ºC for 24 hours. After the incubation period, 40 µl of 0.2 mg/ml Iodonitrotetrazolium Chloride (INT) was added to each of the wells. The microplates were then examined after additional 30 to 120 minutes incubation. Bacterial growth was indicated by INT (yellow colour) being converted to formazan (red colour) and the lowest concentration at which a decrease in the red colour was apparent compared to the next dilution was taken as the MIC value (Eloff, 1998).

The MBC values were deduced from those wells with lowest concentrations at which no growth took place after culture for 24 hours of incubation as described by Nester *et al.*, (2004). Briefly, a small sample from each of those wells was transferred to fresh nutrient agar plates and incubated at 37 ºC for 24 hours and plates examined for the presence or absence of living microorganism. Plates with no microbial growth were regarded as the minimum bactericidal concentrations (Nester *et al.*, 2004).

3.2.11. Pharmacological analysis

3.2.11.1. Acute toxicity (LD_{50}) test

LD_{50} (Lethal Dose) is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material. The test animal used for this test was the Sprague-Dawley rat. The herbal extract was filtered and freeze dried to get the lyophilized extract. Dose levels of 5000mg/kg, 2500mg/kg, and 1250mg/kg of the freeze dried extract were administered orally to the rats per kilogram body weight. The animals were observed for the first 24 hours and then a period of 48 hours for signs of toxicity such as; effect on eyes (eye colour, tears in eyes, bulging), effect on movement (sluggish movement or immobile), effect on

breathing (quick or slow), arrangement of fur (pilo-errection), and twitching gait. General observations other than the above normal behavior were also observed and recorded. The LD_{50} value was expressed as the weight of chemical administered per kilogram body weight of the animal and the values obtained compared to other values by using Hodge and Sterner toxicity scale **(**Lancaster, 2010).

CHAPTER FOUR

4.0. RESULTS

4.1. PHYTOCHEMICAL ANALYSIS

The aqueous and ethanolic extracts of "Antibact" were subjected to phytochemical screening and the results are summarized in Table 1 below. The study revealed the presence of saponins, reducing sugars, phenolics, polyuronides, and triterpenes as the major phytochemical components of both aqueous and ethanolic "Antibact" with alkaloids and flavonoids present only in the ethanolic "Antibact" whilst phytosterols were only present in the aqueous "Antibact".

Table 1. Phytochemical Screening Tests on the Aqueous and Ethanolic extracts of "Antibact"

PLANT CONSTITUENT	ANTIBACT	
	AQUEOUS	ETHANOLIC
Saponins	+	+
Reducing sugars	+	+
Cyanogenic glycoside	-	-
Phenolics	+	+
Polyuronides	+	+
Alkaloids	-	+
Anthracenosides	-	-
Flavonoids	-	+
Phytosterols	+	-
Triterpenes	+	+

4.2. ANTIBIOTIC SUSCEPTIBILITY TESTING OF MICROBES

Twenty four pathogenic microbes were examined for their antibiotic susceptibility. The antibiotics chosen were based on NCCLS standards as well as current treatment regimens for microbial infections in Ghana (NCCLS, 2000). All the test microorganisms were resistant to ampicillin, penicillin, cloxacillin and tetracycline but were variously susceptible or resistant to the rest of the antibiotics used (Table 2). As shown in Table 4 and summarized in Figure 1, all the isolates (100%) were found to be resistant to five or more of the "first line" antibiotics namely; Ampicillin (AMP), Chloramphenicol (CHL), Tetracycline (TET), Gentamicin (GEN), Amikacin (AMK), Cotrimoxazole (COT), Erythromycin (ERY), Penicillin (PEN), Cefixime (CXM), Cefotaxime (CTX), Cefuroxime (CRX) and Cloxacillin (CXC). Thus, all the microorganisms used for this study were multiple resistant that is, resistance to 3 or more antibiotics.

Table 2. Antibiotic Resistance Pattern of the Gram Positive bacteria used.

ANTIBIOTIC	RESISTANT ISOLATES	%
COT	*S. saprophtticus* W1, *S. saprophyticus* W2, *S. aureus* W1, *S. aureus* W2	66.7%
GEN	None of the Gram positive organisms used were resistant to Gentamicin	0%
CXM	*S. saprophtticus* W1, *S. saprophyticus* W2, *S. saprophyticus* ATCC 15305	50%
ERY	*S. saprophtticus* W1, *S. saprophyticus* W2, *S. aureus* W1, *S. aureus* W2	66.7%
TET, CXC, AMP, PEN	All 6 microorganisms used	100%

Table 3. Antibiotic Resistance Pattern of the Gram Negative bacteria used.

ANTIBIOTIC	RESISTANT ISOLATES	%
CRX	***K. pneumoniae W1, K. pneumoniae W2, S. typhi W1, S. typhi W2, S. typhimurium* W1, *S. typhimurium* W2, *P. mirabilis* W1, *E. coli* W1, *E. coli* W2, P. aeruginosa W2, P. aeruginosa ATCC 27853, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 33495, *E. coli* ATCC 25922**	**77.8%**
CTX	***K. pneumoniae W1, K. pneumoniae W2, S. typhi W1, S. typhi W2, E. coli* W1, *E. coli* W2, P. aeruginosa W2, P. aeruginosa ATCC 27853**	**44.4%**
GEN	***S. typhimurium* W2, *S. typhi W1, S. typhi W2, E. coli* W1, *E. coli* W2, *K. pneumoniae W1, K. pneumoniae W2*, P. aeruginosa W2, P. aeruginosa ATCC 27853, *P. mirabilis* ATCC 49565**	**55.6%**
AMK	***S. typhi W1, S. typhi W2, S. typhimurium* W1, *S. typhimurium* W2, *E. coli* W1, *E. coli* W2, *K. pneumoniae W1, K. pneumoniae W2, P. mirabilis* W1, P. aeruginosa W1, P. aeruginosa ATCC 27853, *K. pneumoniae* ATCC 33495, *S. typhimurium* ATCC 14028, *P. mirabilis* ATCC 49565**	**77.8%**
COT	***S. typhi W1, S. typhi W2, S. typhimurium* W1, *E. coli* W1, *E. coli* W2, *K. pneumoniae W1, K. pneumoniae W2, P. mirabilis* W1, *P. mirabilis* W2, P. aeruginosa W1, P. aeruginosa W2, P. aeruginosa ATCC 27853, *K. pneumoniae* ATCC 33495, *S. typhimurium* ATCC 14028, *P. mirabilis* ATCC 49565**	**83.3%**
CHL	***S. typhi W1, S. typhi W2, E. coli* W1, *E. coli* W2, *K. pneumoniae W1, K. pneumoniae W2, P. mirabilis* W1, *P. mirabilis* W2, , *S. typhimurium* W1, *S. typhimurium* W2, P. aeruginosa W1, P. aeruginosa W2, P. aeruginosa ATCC 27853, *S. typhimurium* ATCC 14028**	**77.8%**
TET and AMP	**All 18 microorganisms used**	**100%**

Table 4: Antibiotic resistance among the isolated strains

ANTIBIOTIC	ISOLATE
Resistance to all antibiotics	
CHL, CRX, GEN, COT, TET, AMP, CTX, ERY, AMK, CXC, PEN, CXM	***S. typhi* W1, *S. typhi* W2, *K. pneumoniae* W1, *K. pneumonia* W2, *E. coli* W1, *E. coli* W2, P. aeruginosa ATCC 27853**
Resistance to 10 antibiotics	
CXM, CXC, PEN, ERY, CHL, CRX, COT, TET, AMP, CTX	***S. saprophtticus* W1, *S. saprophyticus* W2**
GEN, CXC, PEN, ERY, CHL, CRX, COT, TET, AMP, CTX	**P. aeruginosa W2**
Resistance to 9 antibiotics	
CXC, PEN, ERY, CHL, CRX, COT, TET, AMP, AMK	***S. typhimurium* W1, *S. typhimurium* ATCC 14028**
GEN, CXC, PEN, ERY, CHL, CRX, TET, AMP, AMK	***S. typhimurium* W2**
CXC, PEN, ERY, CHL, CXM, COT, TET, AMP, AMK	**P. aeruginosa W1**
CXC, PEN, CXM, COT, TET, AMP, AMK, CRX, CTX	***S. saprophyticus* std**
Resistance to 8 antibiotics	
CXC, PEN, COT, TET, AMP, AMK, CHL, ERY	***S. aureus* W1, *S. aureus* W2, *P. mirabilis* W2**
CXC, PEN, COT, TET, AMP, AMK, ERY, CRX,	***K. pneumoniae* ATCC 33495**
CXC, PEN, COT, TET, AMP, CHL, ERY, CRX	***P. mirabilis* W1**
CXC, PEN, COT, TET, AMP, AMK, GEN, ERY	***P. mirabilis* std**
Resistance to 6 antibiotics	
CXC, PEN, TET, AMP, ERY, CRX	***E. coli* ATCC 25922**
CXC, PEN, TET, AMP, CTX, CXM	***S. aureus* ATCC 25923**
Resistance to 5 antibiotics	
CXC, PEN, TET, AMP, ERY	***S. typhi* std**

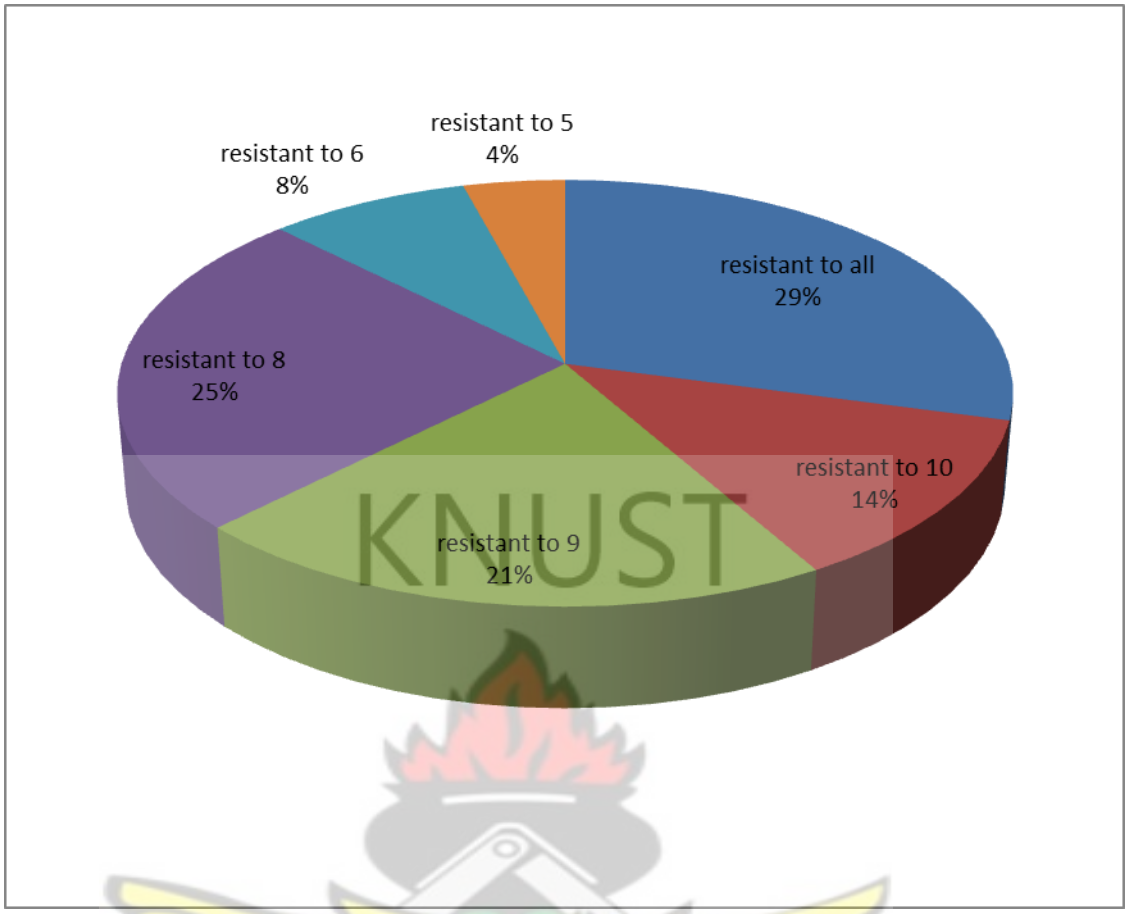

Figure 1: Percentage resistance of the microorganisms to the antibiotics used

4.3. SUSCEPTIBILITY OF MICROORGANISMS TO "ANTIBACT" AND ITS COMPONENT PLANTS

Susceptibility to aqueous and ethanolic extracts of the individual medicinal plants used namely, *P. fraternus, H. opposita, P. guajava* and *C. citratus* as well as a formulation from the above mentioned plants "Antibact" were studies. The isolates that showed zones of inhibition equal to or more than 6mm were considered as susceptible. As shown in Figure 2 and 4, the aqueous extracts of *P. fraternus, H. opposita, P. guajava, C. citrates*, and "Antibact" did not inhibit the growth of both wild and standard strains of *E. coli* and *S. typhimurium*, with the individual plants being able to inhibit *S. typhi* ATCC 19430 (Figure 2) while "Antibact" inhibited the

growth of a single wild strain of *S. typhi* (Figure 4). The ethanolic extracts, on the other hand, inhibited the growth of all the selected human pathogens except *E. coli* ATCC 25922 (Figure 3). From the study, it was also observed that, the aqueous extracts from the individual medicinal plants exhibited wider zones of inhibition against the microorganisms used than the individual ethanolic extracts (Figure 2 and 3). However, the ethanolic extracts from the individual plants inhibited more of the microrganisms used than those from the aqueous extracts. In addition, the ethanolic "Antibact" inhibited more of the microorganisms with wider zones of inhibitions than the aqueous "Antibact" (Figure 4).

4.3.1. Susceptibility of the microorganisms to the aqueous extracts

The susceptibility of microorganisms as well as the antimicrobial activity of the individual aqueous extracts from the four plants was investigated and the results are presented in Tables 5-8 and summarized in Figure 2. Extracts from *P. fraternus* inhibited the growth of 5 out of 7 (71.4%) standard strains with zones of inhibition ranging from 0.0 to 29.67 ± 0.33mm while in the case of the wild strains, growth of 7 out of 14 (50%) strains were inhibited with zones of inhibition ranging from 0.0 to 14.33 ± 0.33mm. It was also observed that all the Gram positive bacteria (100%) were inhibited by *P. fraternus* whilst only 5 out of 15 (33%) Gram negative bacteria were inhibited (Table 5). Thus, the growth of a total of 12 out of 21 (57%) microorganisms used were inhibited by the extract from *P. fraternus* with an average zone of inhibition of 9.37 ± 2.17mm

In the case of *H. opposita*, the growth of 4 out of 7 (57%) standard strains were inhibited with zones of inhibition ranging from 0.0 to 23.00 ± 2.52mm whilst only 1

out of 14 (7%) wild strains were inhibited with zones of inhibition ranging from 0.00 to 4.67 ± 2.33mm. *H. opposita* inhibited the growth of 3 out of 6 (50%) Gram positive bacteria with only 1 out of 15 (7%) Gram negative bacteria inhibited (Table 6). *H. opposita* inhibited the growth of 5 out of 21 (24%) microorganism used averaging 3.17± 1.48mm.

P. guajava inhibited the growth of 5 out of 7 (71%) standard strains with zones of inhibition ranging from 0.00 to 27.00 ± 0.58mm while it was able to inhibit the growth of 7 out of 14 (50%) wild strains with zones of inhibition ranging from 0.00 to 13.67 ± 0.33mm. All 6 (100%) Gram positive bacteria were inhibited while 6 out of 15 (40%) Gram negative bacteria were inhibited by *P. guajava* (Table 7). A total of 12 out of 21 (57%) microorganisms were inhibited by *P. guajava* with an average zone of inhibition of 7.72 ± 1.79mm.

C. citratus did not inhibit any of the microorganisms used (Table 8).

Table 5: Susceptibility of the microorganisms to aqueous extracts of *Phyllanthus fraternus*

Human pathogen	*P. fraternus*							
	Zones of inhibition (mm)				Average deviation	Standard deviation	Standard error of mean	variance
	1	2	3	Mean				
WILD STRAINS								
***S. saprophyticus*(1)**	**14.0**	**12.0**	**10.0**	**12.00**	**1.3**	**2.00**	**1.15**	**4.00**
***S. saprophyticus*(2)**	**14.0**	**15.0**	**14.0**	**14.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis*(1)**	**10.0**	**10.0**	**10.0**	**10.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**14.0**	**15.0**	**10.0**	**13.00**	**2.0**	**2.65**	**1.53**	**7.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**16.0**	**12.0**	**16.0**	**14.67**	**1.8**	**2.31**	**1.33**	**5.33**
***S. aureus*(1)**	**10.0**	**12.0**	**12.0**	**11.33**	**0.9**	**1.15**	**0.67**	**1.33**
***S. aureus*(2)**	**10.0**	**10.0**	**7.0**	**9.00**	**1.3**	**1.73**	**1.00**	**3.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**29.0**	**30.0**	**30.0**	**29.67**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis* ATCC 49565**	**22.0**	**21.0**	**23.0**	**22.00**	**0.7**	**1.00**	**0.58**	**1.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
P. aeruginosa* *ATCC 27853	**22.0**	**26.0**	**27.0**	**25.00**	**2.0**	**2.65**	**1.53**	**7.00**
S. aureus* *ATCC 25923	**27.0**	**27.0**	**26.0**	**26.67**	**0.4**	**0.58**	**0.33**	**0.33**
E. coli* *ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**

Table 6: Susceptibility of the microorganisms to the aqueous extracts of *Hoslundia opposita*

Human pathogen	*H. opposita*							
	Zones of inhibition (mm)							
	1	2	3	Mean	Average deviation	Standard deviation	Standard error of mean	variance
WILD STRAINS								
***S. saprophyticus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. saprophyticus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**7.0**	**7.0**	**0.0**	**4.67**	**3.1**	**4.04**	**2.33**	**16.33**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**19.0**	**20.0**	**20.0**	**19.67**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis* ATCC 49565**	**8.0**	**8.0**	**8.0**	**8.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
P. aeruginosa* *ATCC 27853	**25.0**	**26.0**	**18.0**	**23.00**	**3.3**	**4.36**	**2.52**	**19.00**
S. aureus* *ATCC 25923	**12.0**	**11.0**	**11.0**	**11.33**	**0.4**	**0.58**	**0.33**	**0.33**
E. coli* *ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**

Table 7: Susceptibility of the microorganisms to the aqueous extracts of *Psidium guajava*

Human pathogen	*P. guajava*							
	Zones of inhibition (mm)			Mean	Average deviation	Standard deviation	Standard error of mean	variance
	1	2	3					
WILD STRAINS								
***S. saprophyticus*(1)**	**12.0**	**12.0**	**10.0**	**11.33**	**0.9**	**1.15**	**0.67**	**1.33**
***S. saprophyticus*(2)**	**12.0**	**12.0**	**13.0**	**12.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis*(1)**	**8.0**	**8.0**	**7.0**	**7.67**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis*(2)**	**9.0**	**10.0**	**10.0**	**9.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**11.0**	**11.0**	**7.0**	**9.67**	**1.8**	**2.31**	**1.33**	**5.33**
***S. aureus*(1)**	**8.0**	**9.0**	**7.0**	**8.00**	**0.7**	**1.00**	**0.58**	**1.00**
***S. aureus*(2)**	**13.0**	**14.0**	**14.0**	**13.67**	**0.4**	**0.58**	**0.33**	**0.33**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**26.0**	**27.0**	**28.0**	**27.00**	**0.7**	**1.00**	**0.58**	**1.00**
***P. mirabilis* ATCC 49565**	**16.0**	**15.0**	**16.0**	**15.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
P. aeruginosa* *ATCC 27853	**15.0**	**15.0**	**15.0**	**15.00**	**0.0**	**0.00**	**0.00**	**0.00**
S. aureus* *ATCC 25923	**24.0**	**23.0**	**23.0**	**23.33**	**0.4**	**0.58**	**0.33**	**0.33**
E. coli* *ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**9.0**	**8.0**	**9.0**	**8.67**	**0.4**	**0.58**	**0.33**	**0.33**

Table 8: Susceptibility of the microorganisms to the aqueous extracts of *Cymbopogon citratus*

Human pathogen	*C. citratus*							
	Zones of inhibition (mm)							
	1	2	3	Mean	Average deviation	Standard deviation	Standard error of mean	variance
WILD STRAINS								
***S. saprophyticus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. saprophyticus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis* ATCC 49565**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
P. aeruginosa* *ATCC 27853	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
S. aureus* *ATCC 25923	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
E. coli* *ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

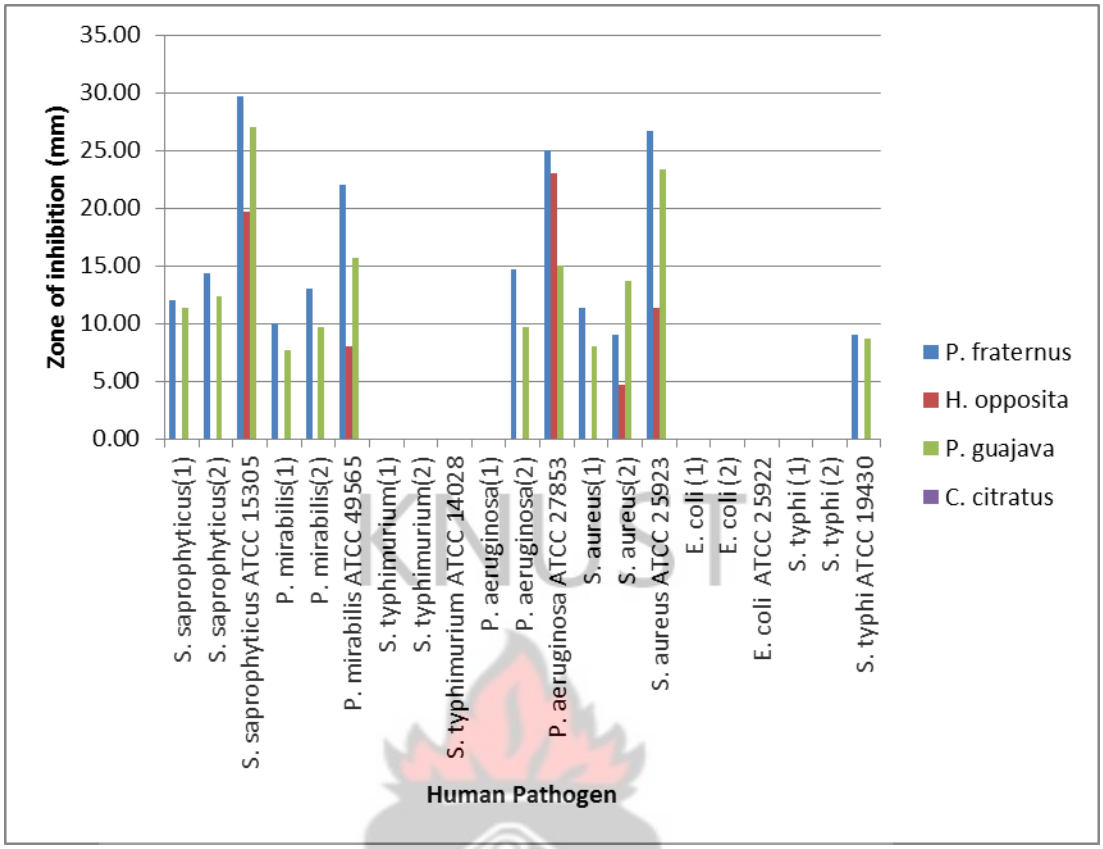

Figure 2. Antimicrobial activity of aqueous extracts of the four individual plants against the twenty one pathogenic microorganisms

4.3.2. Susceptibility of the microorganisms to the ethanol extracts

Results for the susceptibility of microorganisms to the ethanol portions of the individual plants are shown in Tables 9-12 and summarized in Figure 3 below. The ethanolic extracts from *P. fraternus* inhibited the growth of 4 out of 7 (57%) standard strains with zones of inhibition ranging from 0.0 to 18.33 ± 3.33mm while in the case of the wild strains, growth of 6 out of 14 (43%) strains were inhibited with zones of inhibition ranging from 0.0 to 10.00 ± 0.58mm. It was also observed that 4 out of 6 (67%) Gram positive bacteria were inhibited by *P. fraternus* whilst only 6 out of 15 (40%) Gram negative bacteria were inhibited (Table 9). A total of 10 out of 21

(48%) microorganisms investigated were inhibited by the extracts from *P. fraternus* with an average zone of inhibition of 5.25 ± 1.35mm.

In the case of *H. opposita*, the growth of 2 out of 7 (29%) standard strains were inhibited with zones of inhibition ranging from 0.0 to 10.67 ± 0.67mm whilst only 1 out of 14 (7%) wild strains were inhibited with zones of inhibition ranging from 0.00 to 5.33 ± 2.73mm. *H. opposita* inhibited the growth of 3 out of 6 (50%) Gram positive bacteria but did not inhibit the Gram negative bacteria investigated (Table 10). *H. opposita* inhibited the growth of a total of 3 out of 21 (14.3%) microorganisms used with an average zone of inhibition of 1.13 ± 0.64mm.

P. guajava inhibited the growth of 4 out of 7 (57%) standard strains with zones of inhibition ranging from 0.00 to 18.33.00 ± 0.33mm while it was able to inhibit the growth of 11 out of 14 (79%) wild strains with zones of inhibition ranging from 0.00 to 15.33 ± 0.33mm. All 6 (100%) Gram positive bacteria were inhibited while 9 out of 15 (60%) Gram negative bacteria were inhibited by *P. guajava* (Table 11). *P. guajava* inhibited the growth of a total of 15 out of 21 (71.4%) microorganisms used with an average zone of inhibition of 8.79 ± 1.37mm. *C. citrates* did not inhibit any of the microorganisms used (Table 12).

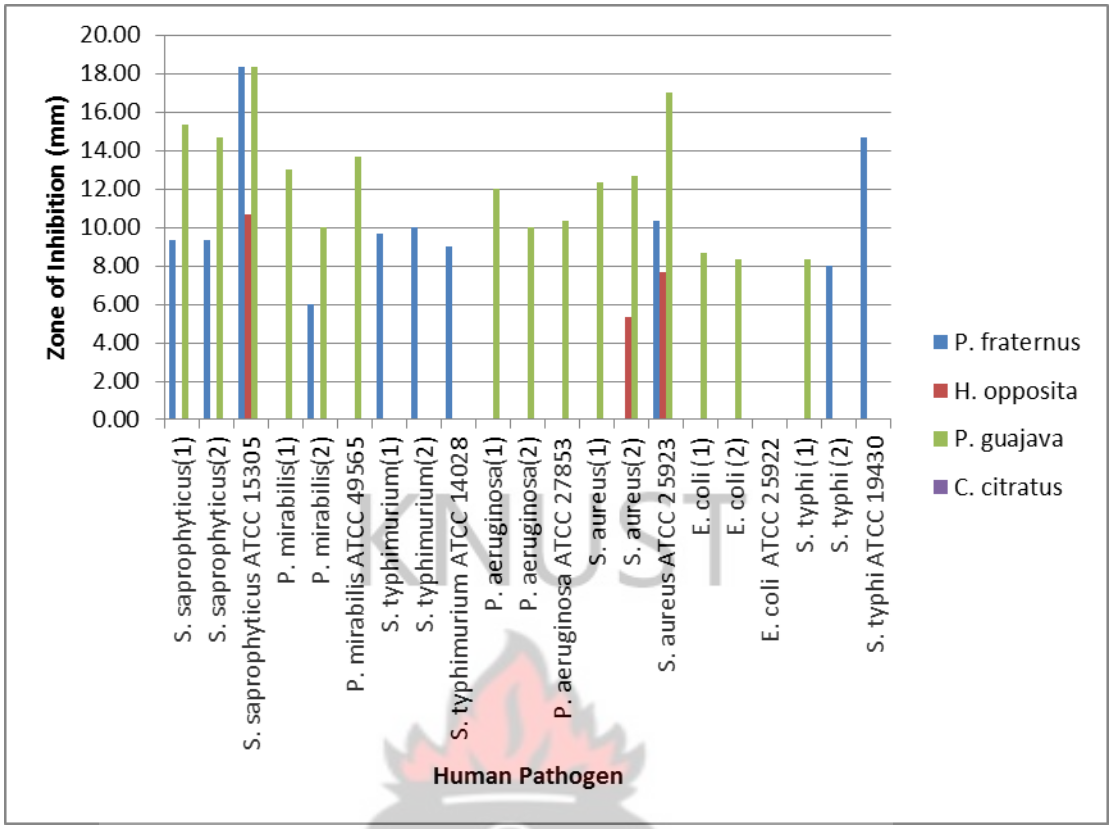

Figure 3. Antimicrobial activity of ethanolic extracts of the four individual plants against the twenty one pathogenic microorganisms

Table 9: Susceptibility of the microorganisms to the of ethanol extracts from *P. fraternus*

Human pathogen	*P. fraternus*							
	Zones of inhibition (mm)				Average deviation	Standard. deviation	Standard error of mean	variance
	1	2	3	Mean				
WILD STRAINS								
***S. saprophyticus*(1)**	**9.0**	**9.0**	**10.0**	**9.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. saprophyticus*(2)**	**10.0**	**10.0**	**8.0**	**9.33**	**0.9**	**1.15**	**0.67**	**1.33**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**8.0**	**10.0**	**0.0**	**6.00**	**4.0**	**5.29**	**3.06**	**28.00**
***S. typhimurium*(1)**	**9.0**	**10.0**	**10.0**	**9.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhimurium*(2)**	**11.0**	**10.0**	**9.0**	**10.00**	**0.7**	**1.00**	**0.58**	**1.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**8.0**	**8.0**	**8.0**	**8.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**15.0**	**25.0**	**15.0**	**18.33**	**4.4**	**5.77**	**3.33**	**33.33**
***S. typhimurium* ATCC 14028**	**15.0**	**14.0**	**15.0**	**14.67**	**0.4**	**0.58**	**0.33**	**0.33**
***E. coli* ATCC 25922**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus* ATCC 25923**	**11.0**	**10.0**	**10.0**	**10.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. aeruginosa* ATCC 27853**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis* ATCC 49565**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**15.0**	**14.0**	**15.0**	**14.67**	**0.4**	**0.58**	**0.33**	**0.33**

Table 10: Susceptibility of the microorganisms to the ethanol extracts from *H. opposita*

Human pathogen	*H. opposita*							
	Zones of inhibition (mm)				Average deviation	Standard deviation	Standard error of mean	variance
	1	2	3	Mean				
WILD STRAINS								
***S. saprophyticus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. saprophyticus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**7.0**	**0.0**	**9.0**	**5.33**	**3.6**	**4.73**	**2.73**	**22.33**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**10.0**	**12.0**	**10.0**	**10.67**	**0.9**	**1.15**	**0.67**	**1.33**
***P. mirabilis* ATCC 49565**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
P. aeruginosa* *ATCC 27853	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
S. aureus* *ATCC 25923	**8.0**	**7.0**	**8.0**	**7.67**	**0.4**	**0.58**	**0.33**	**0.33**
E. coli* *ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**

Table 11: Susceptibility of the microorganisms to the ethanol extracts from *Psidium guajava*

Human pathogen	*P. guajava*							
	Zones of inhibition (mm)				Average deviation	Standard deviation	Standard error of mean	variance
	1	2	3	Mean				
WILD STRAINS								
***S. saprophyticus*(1)**	**16.0**	**15.0**	**15.0**	**15.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. saprophyticus*(2)**	**14.0**	**15.0**	**15.0**	**14.67**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis*(1)**	**13.0**	**12.0**	**14.0**	**13.00**	**0.7**	**1.00**	**0.58**	**1.00**
***P. mirabilis*(2)**	**10.0**	**10.0**	**10.0**	**10.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**11.0**	**12.0**	**13.0**	**12.00**	**0.7**	**1.00**	**0.58**	**1.00**
***P. aeruginosa*(2)**	**10.0**	**10.0**	**10.0**	**10.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**13.0**	**12.0**	**12.0**	**12.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. aureus*(2)**	**13.0**	**12.0**	**13.0**	**12.67**	**0.4**	**0.58**	**0.33**	**0.33**
***E. coli* (1)**	**9.0**	**8.0**	**9.0**	**8.67**	**0.4**	**0.58**	**0.33**	**0.33**
***E. coli* (2)**	**8.0**	**9.0**	**8.0**	**8.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhi* (1)**	**9.0**	**8.0**	**8.0**	**8.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**18.0**	**19.0**	**18.0**	**18.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis* ATCC 49565**	**13.0**	**14.0**	**14.0**	**13.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa* ATCC 27853**	**10.0**	**10.0**	**11.0**	**10.33**	**0.4**	**0.58**	**0.33**	**0.33**
***S. aureus* ATCC 25923**	**16.0**	**18.0**	**17.0**	**17.00**	**0.7**	**1.00**	**0.58**	**1.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* ATCC 25922**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**

Table 12: Susceptibility of the microorganisms to the ethanol extracts from *C. citrates*

	C. citrates							
	Zones of inhibition (mm)							
Human pathogen	**1**	**2**	**3**	**Mean**	Average deviation	Standard deviation	Standard error of mean	variance
WILD STRAINS								
***S. saprophyticus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. saprophyticus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. mirabilis* ATCC 49565**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***P. aeruginosa* ATCC 27853**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***S. aureus* ATCC 25923**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
***E. coli* ATCC 25922**	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
S. typhi* *ATCC 19430	**0.0**	**0.0**	**0.0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

4.3.3. Susceptibility of microorganisms to aqueous and ethanolic "Antibact"

"Antibact" is a formulation from four of the selected plants namely *P. fraternus*, *H. opposita*, *P. guajava* and *C. citrates*. The antimicrobial activity of the formulation was investigated using fourteen clinical isolates and seven standard strains and the results are as shown in Tables 13 and 14 as well as Figure 4.

The aqueous "Antibact" inhibited the growth of 3 out of 7 (43%) standard strains with zones of inhibition ranging from 0.0 to 9.67 ± 0.33mm while only 2 (14%) wild strains were inhibited with zones of inhibition ranging from 0.00 to 10.33 ± 0.33mm. The aqueous "Antibact" also inhibited 4 out of 6 (67%) Gram-positive bacteria while only 1 out of 14 (7%) Gram-negative bacteria were inhibited (Table 12). In total, the aqueous "Antibact" inhibited the growth of 5 out of 21 (23.8%) microorganism used with an average zone of inhibition of 2.32 ± 0.93mm. In the case of the ethanol "Antibact", the growth of 4 out of 7 (57%) standard strains were inhibited with zones of inhibition ranging from 0.00 to 14.00 ± 0.00mm whilst 9 out of 14 (64%) wild strains were inhibited with zones of inhibition ranging from 0.00 to 16.00 ± 1.00mm. The ethanol extract inhibited all 6 (100%) Gram-positive bacteria and 9 of 14(60%) Gram-negative bacteria (Table 13). Ethanolic "Antibact" inhibited the growth of a total of 13 out of 21 (62%) microorganisms used with an average zone of inhibition of 6.68 ± 1.26 mm.

Table 13: Susceptibility of the microorganisms to the aqueous Antibact

Human Pathogen	Zones of inhibition (mm) 1	2	3	mean	Average deviation	Standard. deviation	Standard error of mean	variance
WILD STRAINS								
***S. saprophyticus*(1)**	**10.0**	**10.0**	**10.0**	**10.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. saprophyticus*(2)**	**11.0**	**10.0**	**10.0**	**10.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**9.0**	**10.0**	**10.0**	**9.67**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis* ATCC 49565**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa* ATCC 27853**	**10.0**	**9.0**	**10.0**	**9.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. aureus* ATCC 25923**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* ATCC 25922**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**

Table 14: Susceptibility of the microorganisms to the ethanol "Antibact "

HUMAN PATHOGENS	Zones of inhibition (mm)				Average deviation	Standard. deviation	Standard error of Mean	Variance
	1	2	3	mean				
WILD STRAINS								
***S. saprophyticus*(1)**	**13.0**	**12.0**	**13.0**	**12.67**	**0.4**	**0.58**	**0.33**	**0.33**
***S. saprophyticus*(2)**	**12.0**	**15.0**	**13.0**	**13.33**	**1.1**	**1.53**	**0.88**	**2.33**
***P. mirabilis*(1)**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. mirabilis*(2)**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium*(2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***P. aeruginosa*(1)**	**8.0**	**8.0**	**6.0**	**7.33**	**0.9**	**1.15**	**0.67**	**1.33**
***P. aeruginosa*(2)**	**9.0**	**8.0**	**6.0**	**7.67**	**1.1**	**1.53**	**0.88**	**2.33**
***S. aureus*(1)**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. aureus*(2)**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (1)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***E. coli* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* (1)**	**18.0**	**15.0**	**15.0**	**16.00**	**1.3**	**1.73**	**1.00**	**3.00**
***S. typhi* (2)**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
STANDARD STRAINS								
***S. saprophyticus* ATCC 15305**	**13.0**	**14.0**	**13.0**	**13.33**	**0.4**	**0.58**	**0.33**	**0.33**
***P. mirabilis* ATCC 49565**	**9.0**	**9.0**	**9.0**	**9.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhimurium* ATCC 14028**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
***S. typhi* ATCC 19430**	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
S. aureus ATCC 25923	**14.0**	**14.0**	**14.0**	**14.00**	**0.0**	**0.00**	**0.00**	**0.00**
E. coli ATCC 25922	**0.0**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.00**	**0.00**
P. aeruginosa ATCC 27853	**13.0**	**10.0**	**10.0**	**11.00**	**1.3**	**1.73**	**1.00**	**3.00**

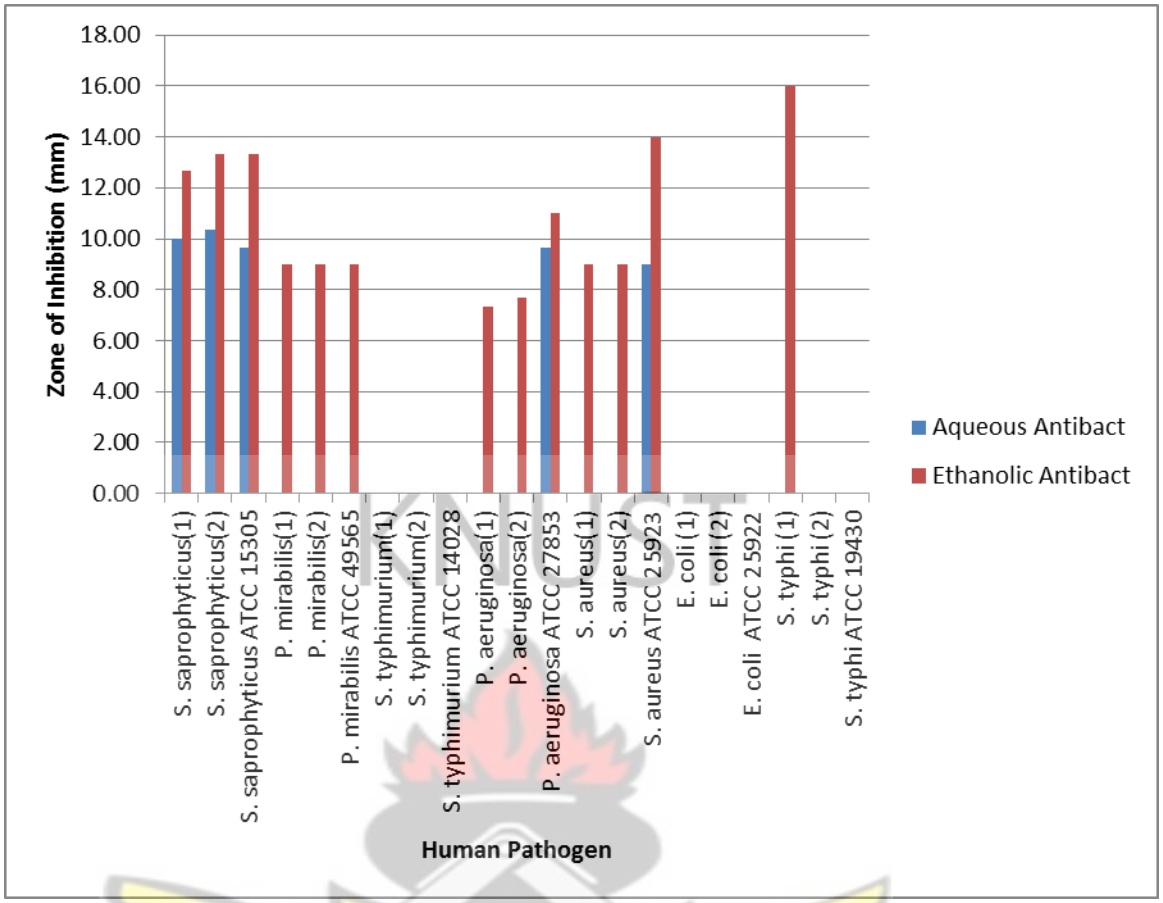

Figure 4. Antimicrobial activity of Aqueous and Ethanol "Antibact" against the twenty one pathogenic microorganisms

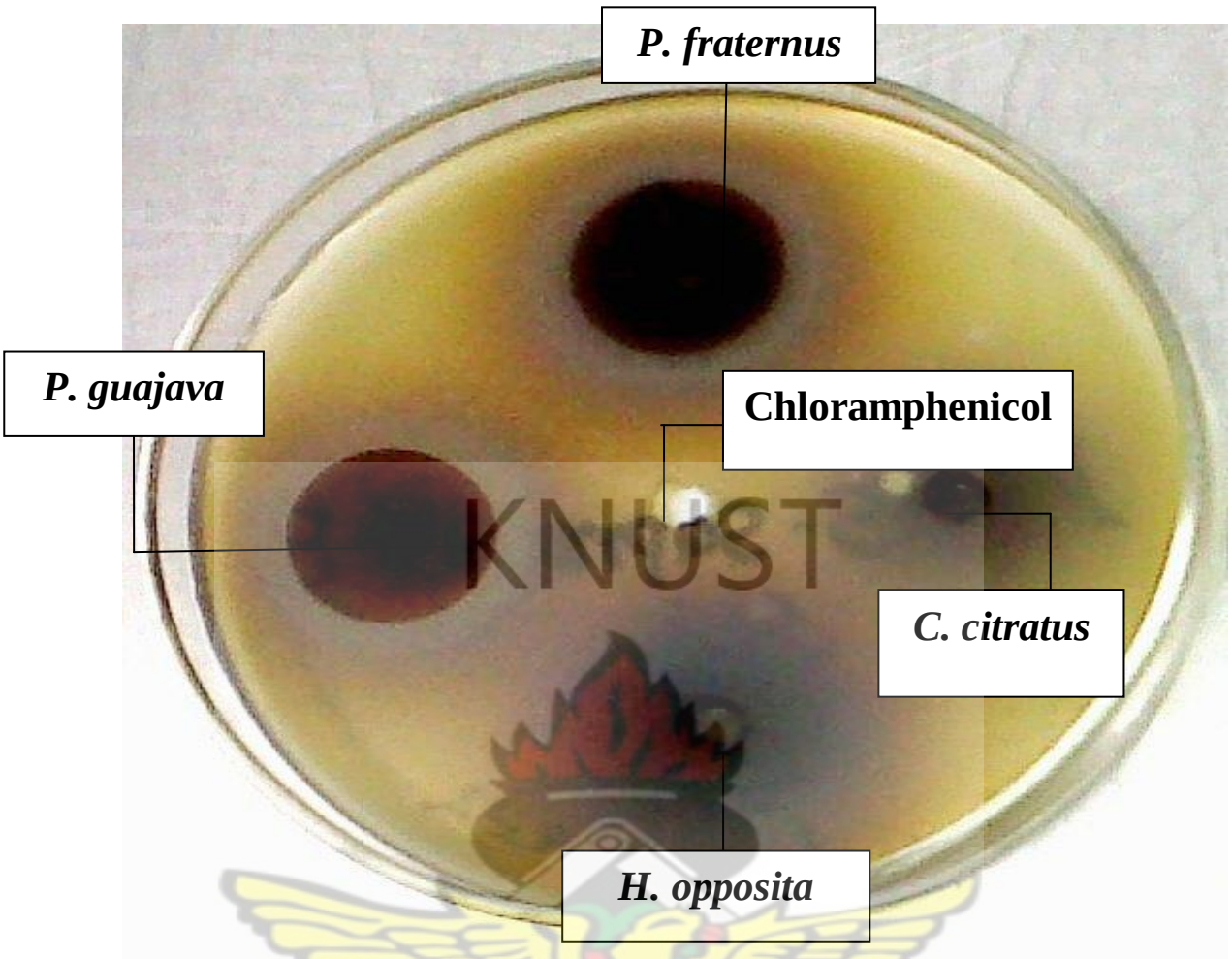

Figure 5: A representative plate showing zones of inhibition exhibited by *P. fraternus*, *H. opposita*, *P. guajava* and *C. citrates* against wild type *S. saprophyticus*

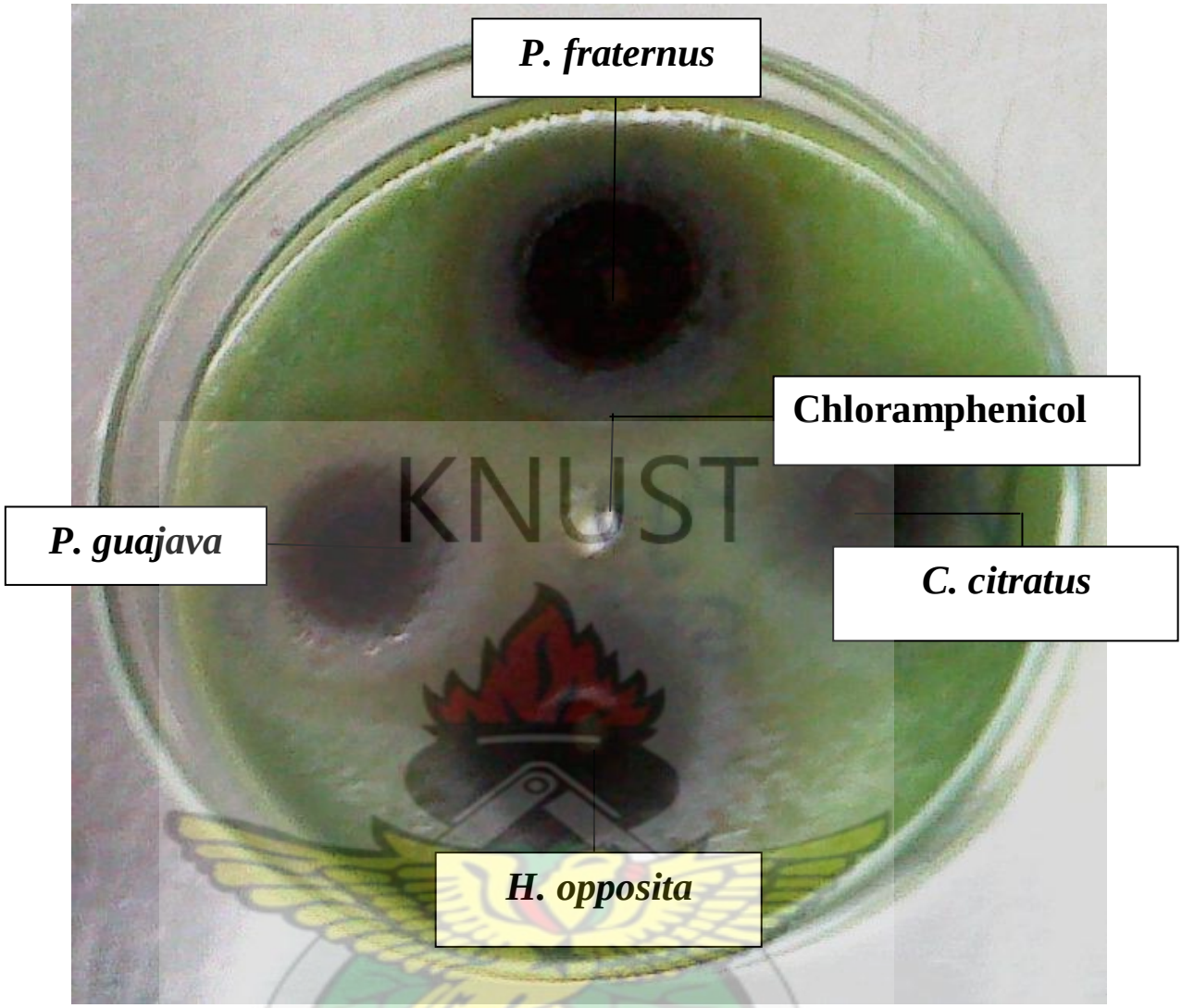

Figure 6: A representative plate showing zones of inhibition exhibited by *P. fraternus*, *H. opposita*, *P. guajava* and *C. citrates* against *P. aeruginosa* ATCC 27853

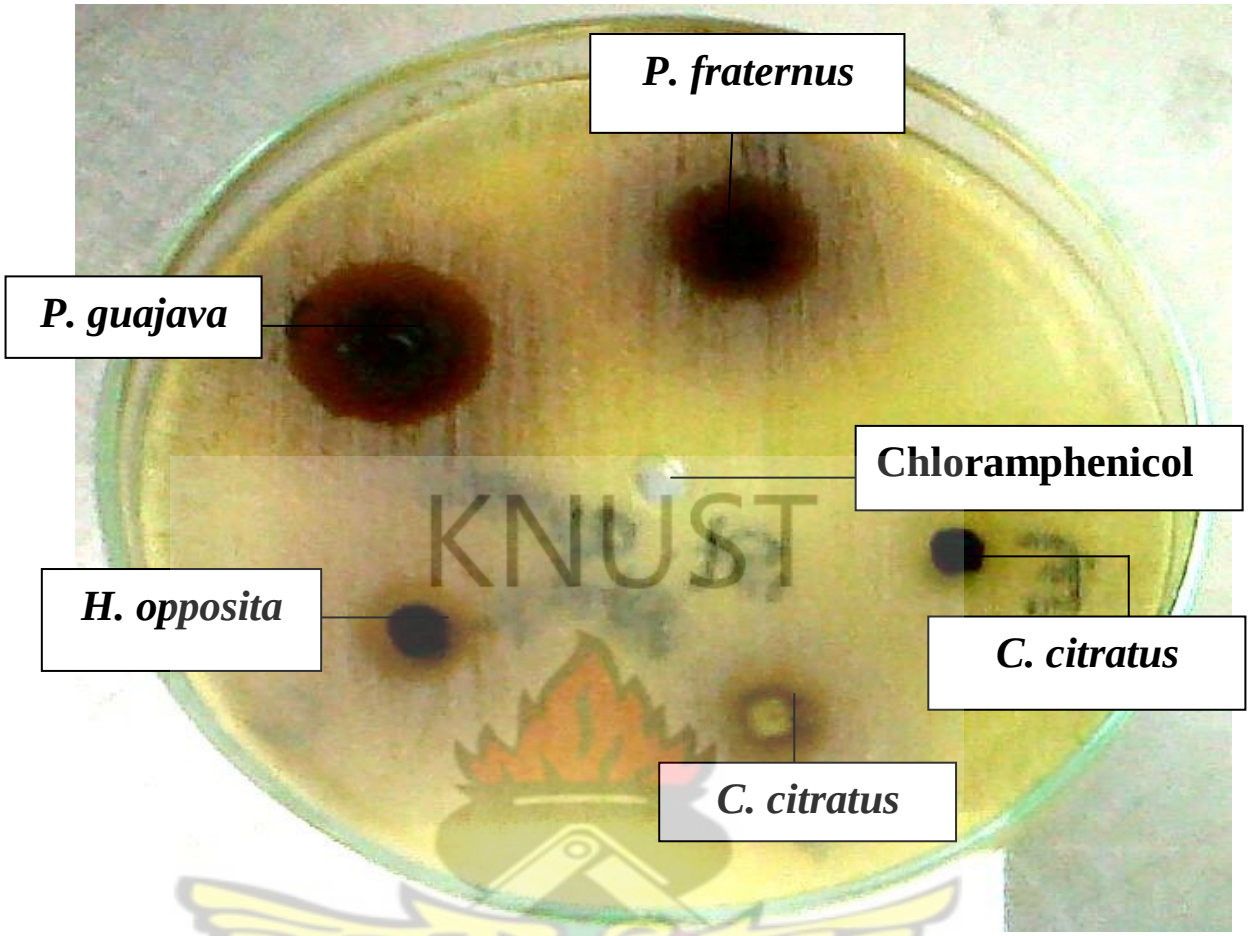

Figure 7: A representative plate showing zones of inhibition exhibited by *P. fraternus, H. opposita, P. guajava* and *C. citrates* against *S. aureus* ATCC 25923

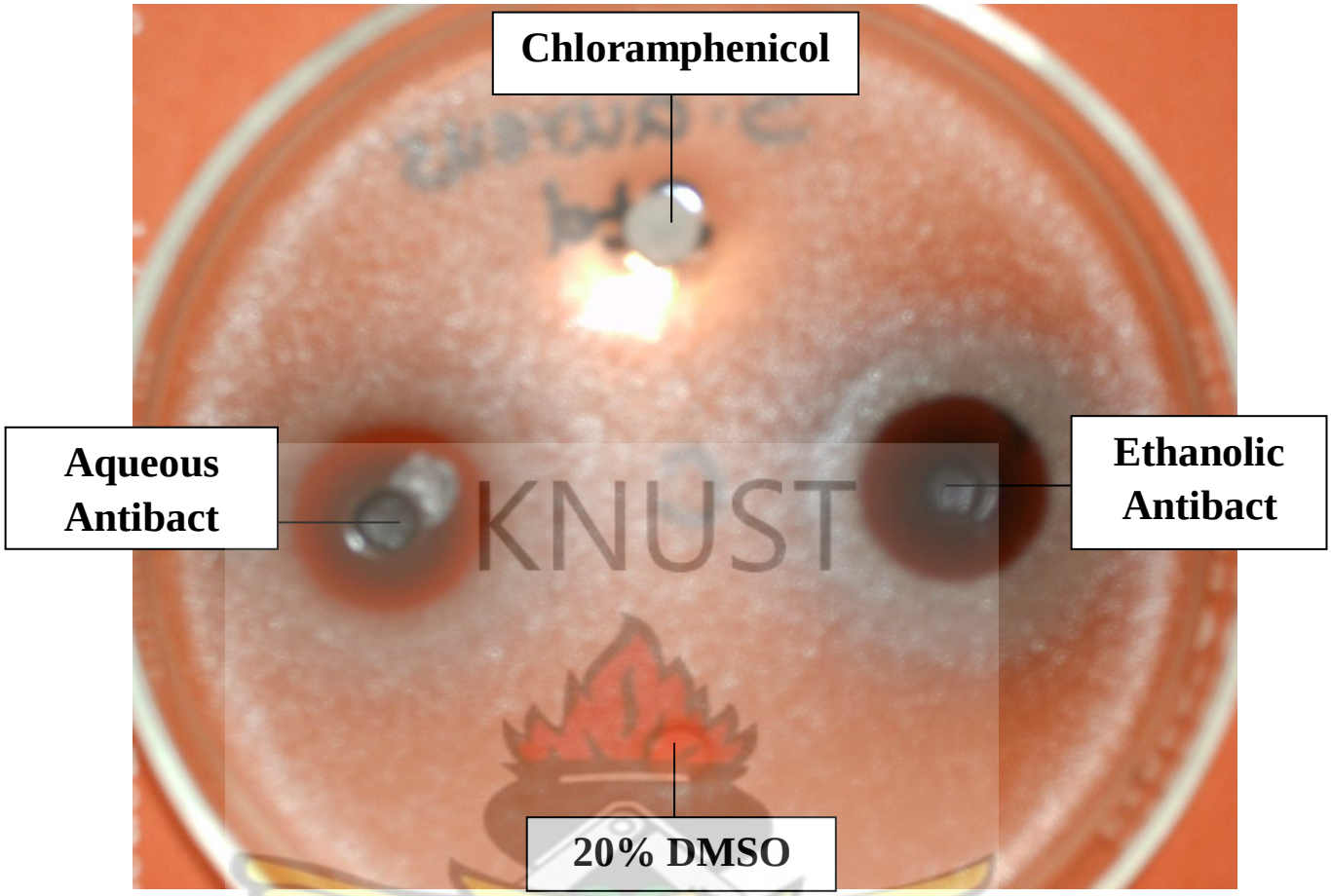

Figure 8: A representative plate showing zones of inhibition exhibited by Antibact against *S. aureus* ATCC 25923

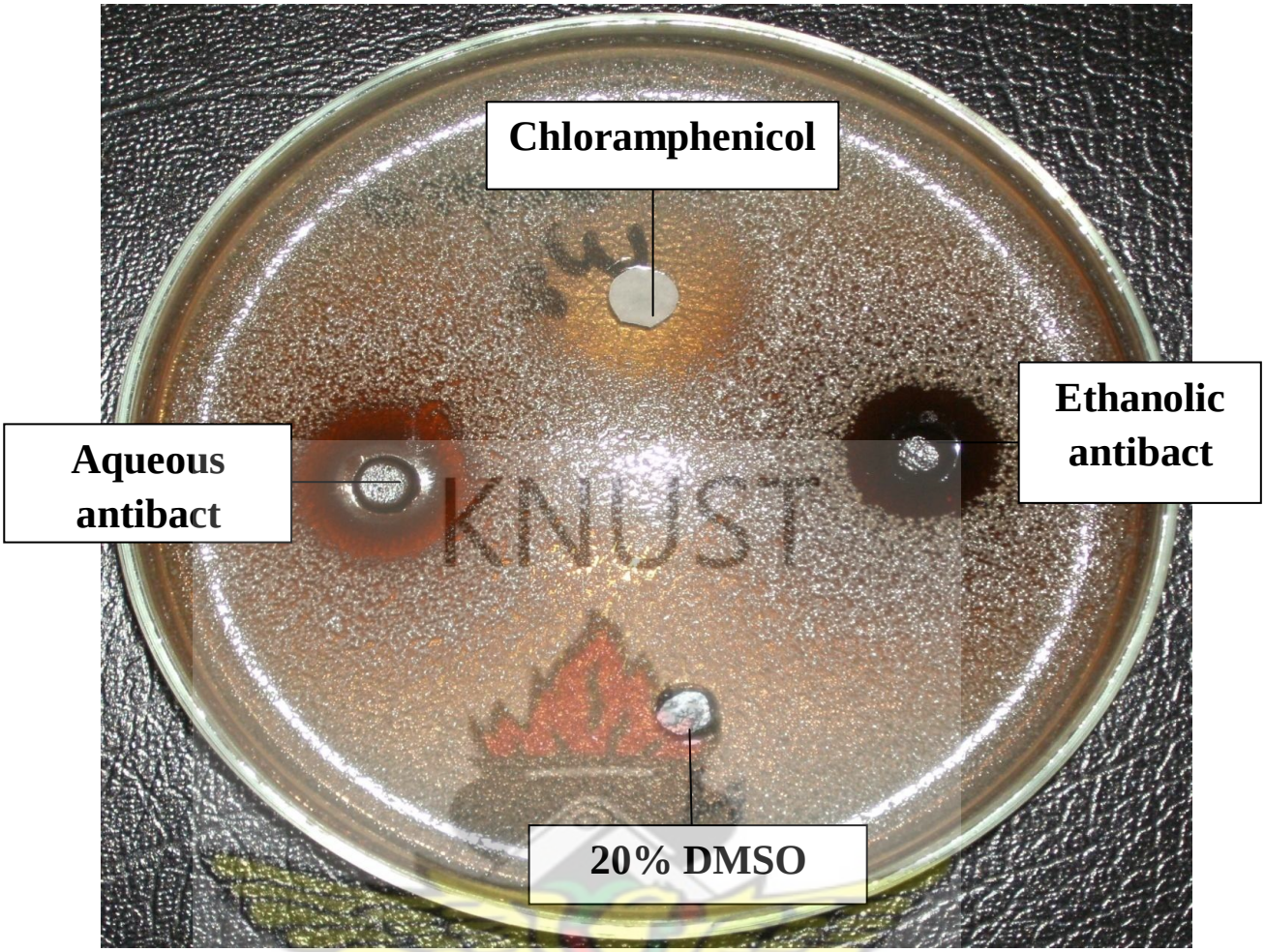

Figure 9: A representative plate showing zones of inhibition exhibited by Antibact against wild type *S. aureus*

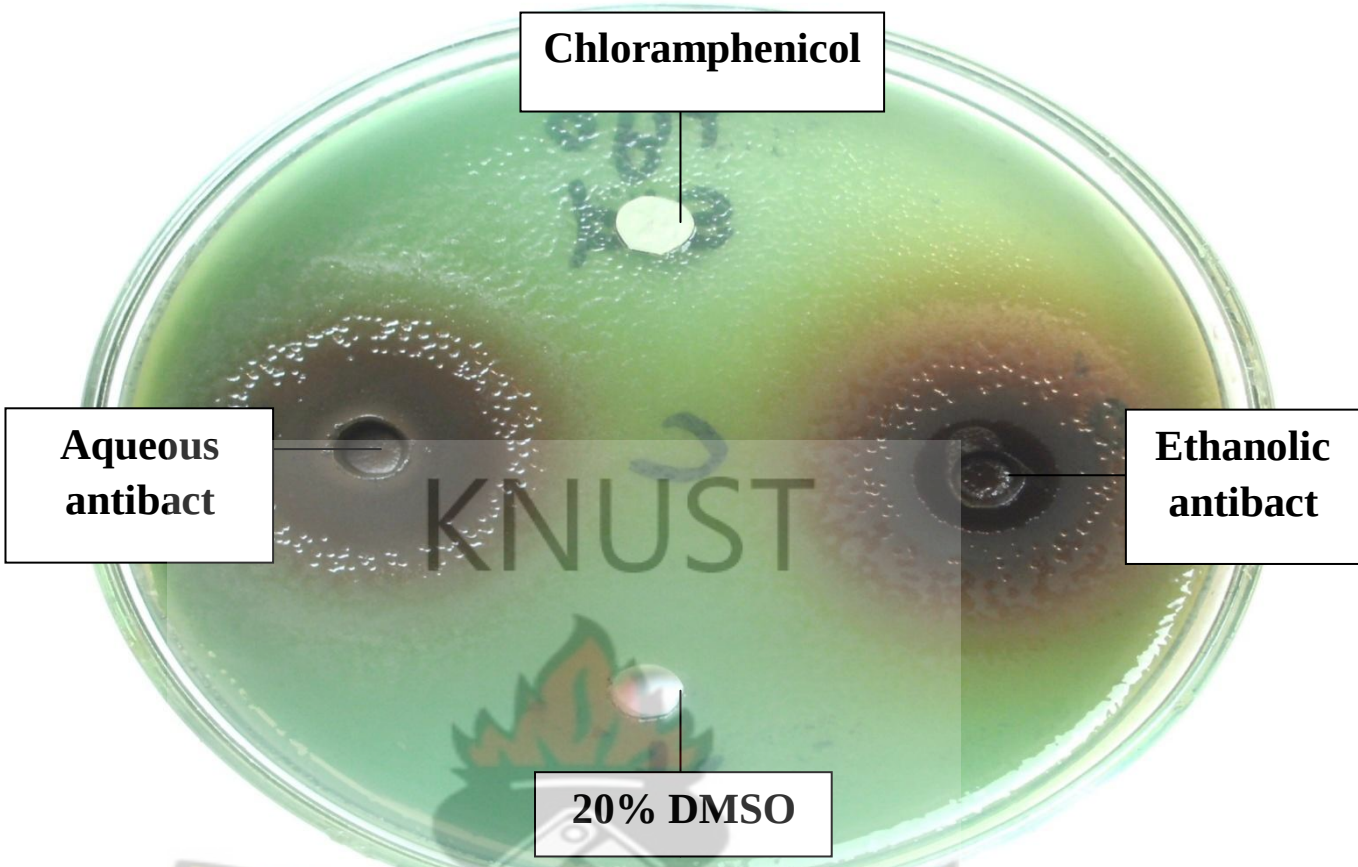

Figure 10: A representative plate showing zones of inhibition exhibited by Antibact against *P. aeruginosa* ATCC 27853

4.4. MICs AND MBCs OF AQUEOUS AND ETHANOL "ANTIBACT"

Results of the MIC and MBC of "Antibact" are summarized in Tables 14-16. Antibacterial effects, expressed as MIC of both ethanolic and aqueous extracts of "Antibact" were observed against *S. saprophyticus, S. aureus, P. aeruginosa, P. mirabilis, S. typhi,* and *E. coli*. For instance, the aqueous "Antibact" exhibited minimum inhibitory concentrations ranging from 0.5 to 16.0 mg/ml for the standard strains whilst the wild strains had MICs ranging from 4.0 to 32.0 mg/ml (Table 15). In the case of the ethanol "Antibact", the MICs ranged between 1.0 and 2.0 mg/ml for the standard strains while for the wild strains it ranged from 2.0 to 8.0 mg/ml (Table 16). The lowest MIC obtained with aqueous extract, was 0.5 mg/ml for *E. coli* ATCC 25922 whereas the highest MIC was 16 mg/mL for wild strain *S. aureus* and *S. saprophyticus.*

Results from the MBC of "Antibact" showed that the aqueous "Antibact" is bacteriostatic in nature while the ethanol "Antibact" is bacteriocidal (Table 18).

Table 15. MICs (mg/ml) of the aqueous extract of Antibact

	MICs mg/ml			
human pathogen	**1**	**2**	**3**	**Average**
WILD STRAINS				
***S. saprophyticus*(1)**	**16.0**	**16.0**	**16.0**	**16.0**
***S. saprophyticus*(2)**	**16.0**	**16.0**	**16.0**	**16.0**
***P. mirabilis*(1)**	**16.0**	**16.0**	**16.0**	**16.0**
***P. mirabilis*(2)**	**16.0**	**16.0**	**16.0**	**16.0**
***P. aeruginosa*(1)**	**4.0**	**4.0**	**4.0**	**4.0**
***P. aeruginosa*(2)**	**32.0**	**32.0**	**32.0**	**32.0**
***S. aureus*(1)**	**16.0**	**16.0**	**16.0**	**16.0**
***S. aureus*(2)**	**16.0**	**16.0**	**16.0**	**16.0**
***E. coli* (1)**	**4.0**	**4.0**	**4.0**	**4.0**
***E. coli* (2)**	**32.0**	**32.0**	**32.0**	**32.0**
***S. typhi* (1)**	**4.0**	**4.0**	**4.0**	**4.0**
***S. typhi* (2)**	**32.0**	**32.0**	**32.0**	**32.0**
STANDARD STRAINS				
S. saprophyticus **ATCC 15305**	**2.0**	**2.0**	**2.0**	**2.0**
P. mirabilis **ATCC 49565**	**4.0**	**4.0**	**4.0**	**4.0**
P. aeruginosa **ATCC 27853**	**8.0**	**8.0**	**8.0**	**8.0**
S. aureus **ATCC 25923**	**16.0**	**16.0**	**16.0**	**16.0**
E. coli **ATCC 25922**	**0.5**	**0.5**	**0.5**	**0.5**
S. typhi **ATCC 19430**	**2.0**	**2.0**	**2.0**	**2.0**

Table 16. MICs (mg/ml) of the ethanol extract of Antibact

	MICs of ethanol Antibact mg/ml			
Human pathogen	**1**	**2**	**3**	**Average**
WILD STRAINS				
***S. saprophyticus*(1)**	**2.0**	**2.0**	**2.0**	**2.0**
***S. saprophyticus*(2)**	**2.0**	**2.0**	**2.0**	**2.0**
***P. mirabilis*(1)**	**4.0**	**4.0**	**4.0**	**4.0**
***P. mirabilis*(2)**	**2.0**	**2.0**	**2.0**	**2.0**
***P. aeruginosa*(1)**	**8.0**	**8.0**	**8.0**	**8.0**
***P. aeruginosa*(2)**	**4.0**	**4.0**	**4.0**	**4.0**
***S. aureus*(1)**	**8.0**	**8.0**	**8.0**	**8.0**
***S. aureus*(2)**	**2.0**	**2.0**	**2.0**	**2.0**
***coli* (1)**	**4.0**	**4.0**	**4.0**	**4.0**
***E. coli* (2)**	**4.0**	**4.0**	**4.0**	**4.0**
***S. typhi* (1)**	**4.0**	**4.0**	**4.0**	**4.0**
***S. typhi* (2)**	**8.0**	**8.0**	**8.0**	**8.0**
STANDARD STRAINS				
S. saprophyticus **ATCC 15305**	**2.0**	**2.0**	**2.0**	**2.0**
P. mirabilis **ATCC 49565**	**2.0**	**2.0**	**2.0**	**2.0**
P. aeruginosa **ATCC 27853**	**2.0**	**2.0**	**2.0**	**2.0**
S. aureus **ATCC 25923**	**1.0**	**1.0**	**1.0**	**1.0**
E. coli **ATCC 25922**	**2.0**	**2.0**	**2.0**	**2.0**
S. typhi **ATCC 19430**	**2.0**	**2.0**	**2.0**	**2.0**

Table 17. MBCs of aqueous and ethanolic extracts of Antibact

	MBCs of 'Antibact'	
human pathogen	**Aqueous**	**Ethanol**
WILD STRAINS		
***S. saprophyticus*(1)**	**32.0**	**8.0**
***S. saprophyticus*(2)**	**32.0**	**8.0**
***P. mirabilis*(1)**	**32.0**	**4.0**
***P. mirabilis*(2)**	**32.0**	**8.0**
***P. aeruginosa*(1)**	**32.0**	**8.0**
***P. aeruginosa*(2)**	**32.0**	**8.0**
***S. aureus*(1)**	**32.0**	**8.0**
***S. aureus*(2)**	**32.0**	**8.0**
***E. coli* (1)**	**32.0**	**16.0**
***E. coli* (2)**	**32.0**	**4.0**
***S. typhi* (1)**	**32.0**	**16.0**
***S. typhi* (2)**	**32.0**	**16.0**
STANDARD STRAINS		
S. saprophyticus **ATCC 15305**	**32.0**	**8.0**
P. mirabilis **ATCC 49565**	**32.0**	**4.0**
P. aeruginosa ***ATCC 27853***	**32.0**	**4.0**
S. aureus ***ATCC 25923***	**320**	**8.0**
E. coli ***ATCC 25922***	**32.0**	**4.0**
S. typhi **ATCC 19430**	**32.0**	**4.0**

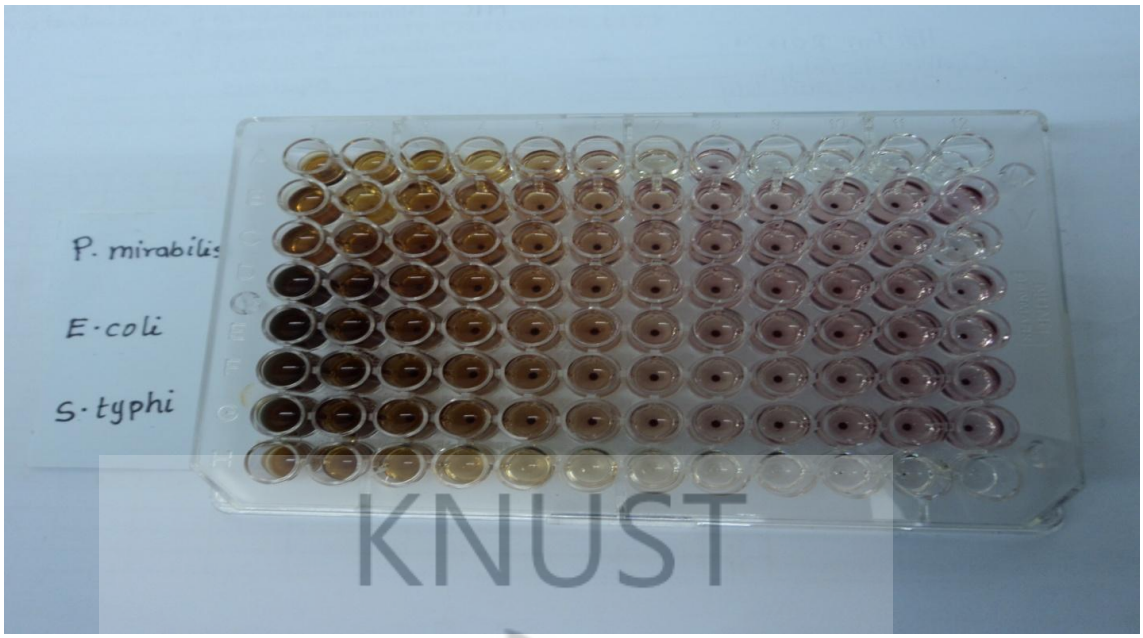

Figure 11: A representative plate showing the MICs of aqueous extract of "Antibact"

Figure 12: A representative plate showing the MIC of ethanol extract of "Antibact"

4.5.PHARMACOLOGICAL ANALYSIS

Determination of LD_{50} seeks to establish the toxicity of "Antibact". The LD_{50} value in this study for both aqueous and ethanol extracts of Antibact is greater than 5000 mg/kg making both herbal medicinal products practically non-toxic according to **Hodge and Sterner Scale (**Lancaster, 2010).

Table 18: Acute toxicity test (LD_{50}) of aqueous and ethanol Antibact extracts

AQUEOUS EXTRACT						
Species and strain	**No. of animals Sex/group**	**Route of administration**	**Formulation and Dosage**	**Time of deaths and period of Observation**	**Approximate. lethal dose (LD_{50})**	**Signs of toxicity**
Sprague-Dawley rats	**12 females; 3 groups (N=4)**	**Oral**	**Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg**	**No death occurred during the period of observation; 48 hours of observation.**	**>5000 mg/kg bodyweight**	**Nil**
ETHANOLIC EXTRACT						
Species and strain	**No. of animals Sex/group**	**Route of administration**	**Formulation and Dosage**	**Time of deaths and period of Observation**	**Approximate lethal dose (LD_{50})**	**Signs of toxicity**
Sprague-Dawley rats	**12 females; 3 groups (N=4)**	**Oral**	**Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg**	**No death occurred during the period of observation; 48 hours of observation.**	**>5000 mg/kg bodyweight**	**Nil**

CHAPTER FIVE

5.0. DISCUSSION

In the present study, the extracts of aqueous and ethanol "Antibact" were subjected to phytochemical screening and the results showed that the herbal medicinal products under study contained saponins, reducing sugars, phenolics, polyuronides, triterpenes, alkaloids flavonoids, and phytosterols (Table 1). These phytochemicals were also found in some of the component plants used to formulate "Antibact".

This study investigated the antimicrobial activities of aqueous and ethanol extracts from all four plants used. Aqueous extract of *P. fraternus* inhibited twelve out of the twenty one (57%) microbes used while the ethanolic extract inhibited ten out of the twenty one (48%). The study revealed that in addition to *P. fraternus* being used to treat hepatitis, cold, flu, tuberculosis, malaria, diabetes and liver diseases among others it has antibacterial activity.

Aqueous and ethanol extracts of *H. opposita* inhibited five (24%) and three (14.3%) out of twenty one microbes used respectively. This study with supporting literature confirms this plant as having antibacterial activity.

Ethanol extract of *P. guajava* inhibited the growth of fifteen (71.4%) of the twenty one test organisms whilst the aqueous extract inhibited twelve (57%) test organisms including standard and wild strains of *S. saprophyticus, P. aeruginosa, P. mirabilis, S. aureus,* and *S. typhi*. This compares with work done by Mills-Robertson, (2004) who demonstrated that ethanol extract of *P. guajava* exhibit wider zone of inhibition

against bacteria than the aqueous extracts. *P. guajava* also inhibited all six (100%) Gram positive bacteria while nine out of fifteen (60%) Gram negative bacteria were inhibited. The aqueous extract of *P. guajava* also exhibited antibacterial property inhibiting all six (100%) Gram positive bacteria while six out of fifteen (40%) Gram negative bacteria were inhibited. In this study, *P. guajava* showed the highest zones of inhibition and degree of antibacterial activity. In spite of this the potential of *P. guajava* in the management of illness is still largely underexplored.

The results of antimicrobial activity of the aqueous and ethanolic extracts of *C. citrates* showed no inhibitory effect on the twenty one pathogenic organisms used in this study. The results obtained was unexpected because the essential oil of *C. citratus* is known to have appreciable activity against bacteria especially Gram positive bacteria (Onawunmi, 1984) whilst several reports also demonstrating the antimicrobial effects of *C. citrates* against animal and plant pathogens, Gram positive and Gram negative bacteria as well as fungus have been documented (Asaolu *et al.,* 2009; Armando and Rahma, 2009). Nwinyi *et al*., (2009) reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Perhaps this non-inhibitory effects produced by the aqueous and ethanol extracts suggest that the active constituents responsible for antiabacterial activity could not be extracted by both the hot boiling water and the ethanol used for the extractions.

This is the first study indicating the effectiveness of "Antibact". The ethanol extract of "Antibact" was more effective than the aqueous extract, inhibiting the growth of thirteen out of twenty one (62%) microbes used while the aqueous "Antibact"

inhibited the growth of a total of five out of twenty one (23.8%) microbes used with an average zone of 2.32 ± 0.93mm and 6.68 ± 1.26mm respectively.

In general, the Gram positive organisms, *S. saprophyticus* and S. aureus were the most susceptible organisms in almost all cases. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieving effect due to the small pores in their cell envelope. The results obtained in this study as far as susceptibility of Gram negative and Gram positive bacteria are concerned compliment other findings of most researchers (Nwiniyi *et al*., 2009; Oskay *et al*., 2009; and Tambekar *et al*., 2009). The most resistant test organism was found to be *K.* pneumoniae that did not show any zone of inhibition to any of the extracts used in this study. This could be due to the capsule, a polysaccharide layer outside the cell wall which is absent in Gram positive bacteria, making it difficult for extracts to penetrate. It has been proposed that the degree of antimicrobial activity of plant extracts against microbes is influenced by conditions such as the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Khan *et al*., 2009).

The low MICs observed during the study indicate that these herbal preparations are efficacious and can be used for the management of diseases caused by the test organisms. The LD_{50} value for "Antibact" is greater than 5000 mg/kg and therefore practically non-toxic according to Hodge and Sterner Scale (Lancaster, 2010). This study has shown that preparations from the individual plants or formulated extracts can be used as effective means for the treatment of infections caused by the human pathogens used in this study.

The observed inhibitory effects of the various herbal extracts in the present study on the tested bacterial isolates is a justification for the need to explore the various traditional modes of disease treatments in order to determine their various antimicrobial efficacies. This is very important as it will assist in standardizing traditional herbal medicaments. Lack of standardization has been described (WHO, 2000) as one of the problems militating against recognition of traditional medicinal practices by orthodox medical practitioners. The antibacterial activities observed in this study are indicative of the presence of some antibacterial compounds and thus this study put forth the importance of preserving and encouraging these medicinal plants according to the standards of scientific achievements and open-evidence-based views toward a more rational policy on medicinal plant research.

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CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

To maintain effective use of antimicrobial drugs in developing countries there has to be improved access to diagnostic laboratories, improved surveillance of the emergence of resistance, better regulation of the use of antibiotics, and better education of the public, doctors, and veterinarians in the appropriate use of the drugs. All of the plant extracts tested in this study with the exception of *Cymbopogon citratus* had potential antibacterial activities against the test strains. This observed difference may be due to insolubility of active compounds in water and ethanol or the presence of inhibitors to the antimicrobial components. Results obtained support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new medicines. From this study, it was observed that the ethanol "Antibact" exhibited high inhibitory activity on the test organisms than the aqueous "Antibact". This could be due to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organisms.

6.2. Recommendation

This study demonstrated the growing concern of multiple resistance to antibiotics especially with all the bacterial strains used during this study being resistant to five or more antibiotics used and the potential of herbal medicine as a suitable alternative. With the evidence of antibacterial activities of the ethanol and water extracts of the herbal preparations studied, it can be rationally suggested that further work needs to

be done to identify and investigate the potential of additional medicinal plant(s) which are known to inhibit the growth of Gram negative bacteria in order to make "Antibact" more effective. It is also important that more species of pathogenic bacteria be tested in order to ascertain the spectra of activities of the antibacterial substances present in the herbal preparations. Further study should involve investigating the phytochemical present in "Antibact" responsible for the antibacterial effect observed in this study and testing of efficacy of "Antibact" using laboratory animals such as mice, guinea pigs and rabbits.

REFERENCES

Abba, D, Inabo, H. I, Yakubu, S. E, And Olonitola, O. S., (2009). Phytochemical Analysis And Antibacterial Activity of Some Powdered Herbal Preparations Marketed in Kaduna Metropolis**.** *Science World Journal;* 4 (1): 23-26

Acharya, D. and Shrivastava, A., (2008). Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices. 1st Edition. Aavishkar Publishers, Distributors, Jaipur, India, ISBN-13: 9788179102527

Addae-Mensah, I. Plant biodiversity, herbal medicine, Intellectual property rights and industrially developing countries: socio-economic, ethical and legal implications. *www.crvp.org/book/Series02/II-5/chapter_vii.htm*

Agyare C, Mensah A Y, and Osei-Asante S., (2006). Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. *Boletin Latinoaericano y del Caribe de Plantas Medicinales y Aromaticas*; 5 (6): 113-117

Ahorlu C. K, Dunyo, S. K, Afari, E. A, Koram, K. A, and Nkrumah, F. K., (1997). Malaria-related beliefs and behaviour in southern Ghana: implications for treatment, prevention and control. *Tropical Medicine and International Health*; 2(5): 488–499

Akah, P.A. and Nwanbie, A.L. (1993): Nigerian plants with anti-convulsant property. *Fitoterapia*; 64(1): 42-44

Akinpelu, D. A and Onakoya, T. M., (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African Journal of Biotechnology*; 5(11): 1078-1081

Alonso-Paz, E, Cerdeiras, M. P, Fernandez, J, Ferreira, F, Moyna, P, Soubes, M, Vazquez, A, Veros, S, and Zunno, L., (1995). Screening of Uruguayan

medicinal plants for antimicrobial activity. *Journal Ethnopharmacology*; 45(1): 67-70

Amadi, E. S, Uzoaru, P. N, Orji, I, Nwaziri, A.A, and Iroha, I. R., (2009). Antibiotic Resistance in Clinical Isolates of *Pseudomonas aeruginosa* in Enugu and Abakaliki, Nigeria . *The Internet Journal of Infectious Diseases;* 7(1): ISSN: 1528-8366

Anesini, E, and Perez, C., (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal Ethnopharmacology*; 39(2): 119-128

Annan, K and Dickson, R., (2008). Evaluation of Wound Healing Actions of *Hoslundia opposita* Vahl, *Anthocleista Nobilis* G. Don. And *Balanites Aegyptiaca* L. *Journal of Science and Technology;* 28(2): 26-38

Antibiotics drug use, monitoring and evaluation of resistance (ADMER) http://admerproject.org/2011

Armando, C. C And Rahma, H. Y., (2009). Evaluation of the yield and the antimicrobial activity ofthe essential oils from: *Eucalyptus Globulus*, *Cymbopogon Citratus* And *Rosmarinus Officinalis* in Mbarara district (Uganda). *Revista Colombiana de Ciencia Animal*; 1(2): 240-249

Asaolu, M. F, Oyeyemi, O. A, and Olanlokun, J. O., (2009). Chemical Compositions, Phytochemical Constituents and *in vitro* Biological Activity of Various Extracts of *Cymbopogon citrates. Pakistan Journal of Nutrition;* 8(12): 1920-1922

Blomberg, B, Manji, K. P, Urassa, W. K, Tamim, B. S, Mwakagile, D. S. M, Jureen, R, Msangi, V, Tellevik, M. J, Holberg-Petersen, M, Harthug, S, Maselle, S. Y, and Langeland, N., (2007). Antimicrobial resistance predicts death in

Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infectious Diseases*; 7: 43. doi:10.1186/1471-2334-7-43

Brusch, J. L, Garvey, T, Corales, R, and Schmitt, S. K., (2010). Typhoid fever. http://emedicine.medscape.com/article/226434-overview (accessed 2011 January 12)

Burkill, H. M., (1985). The useful plants of West Tropical Africa, KWE, published by Royal Botanic Gardens. Entry for Phyllanthus fraternus Webster [family EUPHORBIACEAE]. Volume 2. http://plants.jstor.org/upwta/2_236

Burkill, H. M., (1997). The useful plants of West Tropical Africa. Families M-R. Royal Botanic Gardens Kew. Edition 2. Vol. 4 ISBN No.1-900347-13-X.

Chessbrough M., (2000). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cambridge, UK. Pp 157-158, 188

Chhabra, S.C. and Uiso, F.C. (1991): Antibacterial activity of some Tanzanian plants used in traditional medicine. *Fitoterapia;* 62(6): 499-503

Clinical and Laboratory Standards Institute (CLIS), (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard M7 A4, CLIS, Wayne, PA, USA

Darko, N. I., (2009): Ghanaian Indigenous Health Practices: The Use of Herbs. Graduate Department of Sociology and Equality Studies in Education. University of Toronto, Canada. pp 1-104

Dayie, N. T, Newman, M. J, Ayitey-Smith, E, and Tayman, F. S. K., (2008). Screening for Antimicrobial Activity of *Ageratum conyzoides L.*: A Pharmaco-Microbiological Approach . *The Internet Journal of Pharmacology;* 5(2): ISSN: 1531-2976

Dubey N.K., Kumar R, and Tripathi P., (2004). Global promotion of herbal medicine: Indian's opportunity. *Current Science;* 86: 37-41

Eloff, J. N., (1998). A sensitivity and quick microplate method to determine the minimal inhibition concentration of plant extracts for bacterial, Planta Medicine, Vol. 64(8), pp. 711-713

Elujoba, A.A, Odeleye, O. M and Ogunyemi, C. M., (2005). Traditional Medical Development for medical and dental primary Health care Delivery System in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*; 2(1): 46-61.

Falodun, A,, (2010). Herbal medicine in Africa-distribution, standardization and prospects. *Research Journal of Phytochemistry*; 4(3): 154-161

Fandohan, P, Gnonlonfin, B, Laleye, A, Gbenou, J. D, Darboux, R, and Moudachirou, M., (2008). Toxicity and gastric tolerance of essential oils from *Cymbopogon citrates*, Ocimum gratissimum and Ocimum basilicum in Wistar rats. *Food and Chemical Toxicology*; 46(7): 2493-2497

Franco, B. E, Martínez, M. A, Rodríguez, M. A. S, and Wertheimer, A. I., (2009). The determinants of the antibiotic resistance Process. *Infection and Drug Resistance;* 2 1–11

Gallardo, F, Ruiz, J, Soto, S. M, Jimenez de Anta, M. T, and Vila, J., (2003). Different antibiotic resistance mechanisms associated with integrons in clinical isolates of *Salmonella typhimurium*. Rev Esp Quimioter; 16(4): 398-402

Gibbons S., (2005). Plants as a source of bacterial resistance modulators and anti-infective agents. *Phytochemistry Reviews;* 4(1): 63-78.

Gottlieb, O. R., Borin, M. R, and Brito, N. R. (2002). Integration of ethnobotany and phytochemistry: dream or reality? *Phytochemistry*; 60(2): 145-152.

Gupta, K, Scholes, D, Stamm, W. E., (1999). Increasing Prevalence of Antimicrobial Resistance Among Uropathogens Causing Acute Uncomplicated Cystitis in Women. *Journal of the American Medical Association;* 281(8): 736-738

Hammer KA, Carson CF, and Riley TV, (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal Applied Microbiology*; 86(6): 985-990.

Haq, I., (2004). Safety of medicinal plants. *Pakistan Journal of Medical Science;* Vol. 43(4): 160-165

Hart, C. A. and Kariuki, S., (1998). Antimicrobial resistance in developing countries. *British Medical Journal*; 317(7159): 647-650

Hedberg, I., Hedberg, O., Madah, P.J., Mshigeni, K.E., Mshin, E.N., Sammelson, G. (1983). Inventory of plants used in traditional medicine in Tanzania II. Plants of the Families Dilleniaceae-Opiliaceae. *Journal of Ethnopharmacology*; 9(1): 105-127

Helal, G. A, Sarhan, M. M, Abu Shahla, A. N, and Abou El-Khair, E. K., (2006). Effects of Cymbopogon citratus L. essential oil on the growth, lipid content and morphogenesis of Aspergillus niger ML2-strain. *Journal of Basic Microbiology*; 46(6): 456-469.

Hostettmann, K. and Hamburger, M., (1991). Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*; 30(12): 3864-3874

Iwu, M.M., (1993). Handbook of African Medical plants. CRC press, Boca Raton, Florida, USA. 367pp.

Iwu, M.W, Duncan A. R, and Okunji C. O., (1999). New antimicrobials of plant origin In: Janick, J. (Ed.). Perspective on New Crops and New Uses. ASHS Press, Alexandr. pp: 457-462.

Jaiarj, P, Khoohaswan, P, Wongkrajang, Y, Peungvicha, P, Suriyawong, P, Saraya, M. L. S, and Ruangsomboon, O., (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *Journal of Ethnopharmacology*; 67(2): 203–212

Kapil, A., (2005). The challenge of antibiotic resistance: Need to contemplate. *Indian Journal of Medical Research;* 121(2): 83-91.

Khan, R, Islam, B, Akram, M, Shakil, S, Ahmad, A, Ali, S. M, Siddiqui, M, and Khan, A. U. (2009). Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules;* 14: 586-597

Khanna AK, Rizvi F, Chander R (2002): Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats. *Journal of Ethnopharmacology*; 82(1): 19-22

Kishore, N, Mishra, A. K, and Chansouria, J. P. N., (1993). Fungitoxicity of essential oils against dermatophytes. *Mycoses*; 36(5-6): 211-215.

Kumaraswamy, M. V, Kavitha, H. U and Satish, S., (2008). Antibacterial Evaluation and Phytochemical Analysis of *Betula utilis* D. Don against Some Human Pathogenic Bacteria. *World Journal of Agricultural Sciences;* 4 (5): 661-664

Lancaster, M. (2010). Green Chemistry: An Introductory Text. The Royal Society of Chemistry, Uk. Pp 20

Lima, E. O, Gompertz, O. F, Giesbrecht, A. M, and Paulo, M. Q., (1993). In vitro antifungal activity of essential oils obtained from officinal plants against dermatophytes. *Mycoses*; 36(9-10): 333-336

Lowe, H, Payne-Jackson, A, Beckstrom-Sternberg, S M, and Duke, J A., (2000). Jamaica's Ethnomedicine: Its potential in the healthcare system. Canoe Press, University of the West Indies, Kingston, Jamaica. pp 170

Lowy, F. D., (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *Journal of Clinical Investigation*; 111(9): 1265–1273

Mahamat, A, Lavigne, J. P, Bouziges, N, Daurès, J. P, and Sotto, A., (2006). Antimicrobial susceptibility of Proteus mirabilis urinary tract isolates from 1999 to 2005 at Nîmes University Hospital. *Pathologie Biologie*; 54(8-9): 456-461

Malau, M. B, Titus, M, and Casmir, I. I. C., (2009). Analysis of the phytochemical and *invivo* antimalaria properties of P*hyllanthus fraternus* webster extract. *New York Science Journal*; 2(5): 12-19

Mathias, A.J, Somashekar, R. K, Sumithraand, S, and Subramanya S., (2000). An Assessment of Reservoirs of Multi-resistant Nosocomial Pathogens in a Secondary care hospital. *Indian Journal of Microbiology*; 40: 183-190.

Mills-Robertson, F. C., (2004). Characterization of wild-type *Salmonella* and their susceptibility to "Mist Enterica", An Anti-Typhoid Herbal Preparation. PhD. Thesis, University of Ghana

Mills-Robertson, F. C, Addy, M. E, Mensah, P, and Crupper, S. S., (2002). Molecular characterization of antibiotic resistance in clinical *Salmonella typhi* isolated in Ghana. *FEMS Microbiology Letters*; 215(2): 249-253

Mills-Robertson, F., Crupper, S.S., Addy, M.E., Mensah, P., (2003a): Antibiotic resistance and genotyping of clinical group B *Salmonella* isolated in Accra, Ghana. *Journal of Applied Microbiology*; 94: 289-294

Mills-Robertson, F.C., Newman, M.J., Mensah, P., Addy, M.E., (2003b): Multiple resistant *Salmonella* in Accra, Ghana. *Ghana Medical Journal*; 37(4): 165-168

Mishra, A. K and Dubey, N. K., (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied Environmental Microbiology*; 60(4): 1101-1105

Mills-Robertson, F. C, Aboagye, F. A, Duker-Eshun, G, Kaminta, S and Agbeve, S., (2009). *In vitro* antimicrobial activity of *Cryptolepis sanguinolenta* (periplocaceae). *African Journal of Pharmacy and Pharmacology*; Vol. 3(9): 476-480

Mohana, D. C, Satish, S and Raveesha, K. A., (2008). Antibacterial Evaluation of Some Plant Extracts Against Some Human Pathogenic Bacteria. *Advances in Biological Research*; 2(3-4): 49-55

Montefiore, D, Rotimi, V. O, and Adeyemi-Doro, F. A. B., (1989). The Problem of Bacterial Resistance to Antibiotics among Strains Isolated from Hospital Patients in Lagos and Ibadan, Nigerian. *Journal of Antimicrobial Chemotherapy*; 23(4): 641-651

Mshana N. R, Abbiw D. K, Addae-Mensah I, Adjanohoun E, Ahji M. R. A, Enow-Orock E. G, Gbile Z. O, Odei M. A, Adenlami H, Oteng-Yeboah A. A, Sarppony K, Sofowora A, Tackie A. N., (2000). Traditional medicine and pharmacopoeia contribution to the revision of Ethno botanical and floristic

studies in Ghana, Scientific, Technical and Research Commission of the Organization of African Unity. OAU/STRC. 1-459

Mujovo, S. F., (2009). Antimicrobial activity of compounds isolated from *Lippia javanica* (Burm.f.) Spreng and *Hoslundia opposita* against *Mycobacterim tuberculosis* and HIV-1 reverse transcriptase. PhD thesis, University of Pretoria.

Nair, R and Chanda, S., (2007). *In-vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. Brazilian Journal of Microbiology; 38(3): 452-458

Nascimento, G. G. F, Locatelli, J, Freitas, P. C, and Silva, G. L., (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-Resistant Bacteria. *Brazilian Journal of Microbiology*; 31 (4): 247-256

National Centre for Infectious diseases, Center for Disease Control and prevention, WHO, (1999). Laboratory Methods for Diagnosis of Epidemic Dysentery and Cholera, Center for Disease Control and prevention, Atlanta Georgia, USA, pp 61-72

National Plant Data Center, USA (2008). Cymbopogon citratus (DC. ex Nees) Stapf. Plants Database. (http://plants.usda.gov (accessed 2010 December 11)

Nester, E. W, D. G. Anderson, C. E. Roberts and M. T. Nester (2008). Microbiology, A human perspective, International Edition, MacGraw-Hill, New York, USA, pp 508, 614, 640-641, 509-512

Newman, M J, Frimpong, E, Asamoah-Adu, A, and Sampane-Donkor, E., (2006). Resistance to Antimicrobial Drugs in Ghana - The Ghanaian-Dutch Collaboration for Health Research and Development. pp 1-19

Ngadjui, B.T., Ayafor, J.F., Sondengam, B.L., Connolly, J.D., Rycroft, D.S. (1991): Hoslundin, Hoslundial and Hoslunddiol, three new flavonoids from the *Hoslundia opposita* (Lamiaceae). *Tetrahedron*; 47(22): 3555-3564

Nicollet, L. E AND Harding G. K. M., (1982). Susceptibility of Clinical Isolates of *Staphylococcus saprophytic*us to Fifteen Commonly Used Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy*; 22(5): 895-896

Nwachukwu, I. N, Allison, L.N, Chinakwe, E. C, and Nwadiaro, P., (2008). Studies on the effects *Cymbopogon citratus, Ceiba pentandra* and *Loranthus bengwelensis* extracts on *species of* dermatophytes**.** *The Journal of American Science*; 4(4): 58-67

Nwinyi, O. C, Nwodo C. S, Olayinka A. O, Ikpo C. O, and Ogunniran, K. O., (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus. African Journal of Food Science*; 3(3): 077-081

O'Brien,T. F, Mayer, K. H, Hopkins, J. D, Farrell, J. J, Chao, L, and Kent, R. L., (1985). Resistance to antibiotics in clinical isolates of *Klebsiella pneumoniae*. *Infection Control*; 6(2):64-67

Ogata, T., higuchi, H., Mochida, S., Matsumoto, H., Kato, A., Endo, T., Kaji, A., and Kaji, H. (1992): HIV-1 reverse transcription inhibitor from *Phyllanthus niruri*. *AIDS Research on Human Retroviruses*; 8(11): 1937-1944

Ogunlana, E. O, Höglund, S, Onawunmi, G, and Sköld, O., (1987). Effects of lemongrass oil on the morphological characteristics and peptidoglycan synthesis of Escherichia coli cells. *Microbiology*; 50(202): 43-59

Okeke, I.N, Lamikanra, A, and Edelman, R., (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases*; 5(1): 18-27.

Okigbo, R. N and Mmeka, E. C., (2006). An appraisal of Phytomedicine in Africa *King Mongkut's Institute of Technology Landkrabang Science Journal* (Thailand); 6(2): 83 - 93.

Okigbo, R. N. and Ajalie, A. N., (2005). Inhibition of some Human pathogens with Tropical Plant extracts. *International* Journal of *Molecular Medicine Advanced Sciences*; Pakistan; 1 (1): 34 - 41

Okoli, R. I, Turay, A. A, Mensah, J. K and Aigbe, A. O., (2009). Phytochemical and Antimicrobial Properties of Four Herbs from Edo State, Nigeria. *Report and Opinion*; 1(5): 67-73

Olajide, O. A, Awe, S. O, and Makinde, J. M., (1999), Pharmacological studies on the leaf of *Psidium guajava. Fitoterapia*; 70(1): 25-31

Oliver-Bever B (1986). Medicinal Plants in tropical West Africa. Cambridge University Press, Cambridge. ISBN No. 0-521-26815-X.

Olowe, O.A, Olayemi, A. B, Eniola, K. I. T, and Adeyeba, A. O., (2003). Aetiologic agents of diarrhoea in children under five years of age in Osogbo. *African Journal of Clinical and Experimental Microbiology*; 4(3): 62- 66

Olowe, O. A, Okanlawon, B. M, Olowe, R. A, and Olayemi, A. B., (2008). Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, south western Nigeria. *African Journal of Microbiology Research*; 2: 008-011

Onawunmi, G. O, Yisak, W. A, and Ogunlana, E. O., (1984). Antibacterial constituents in the essential oil of Cymbopogon citratus (DC.) Stapf. Journal Ethnopharmacology; 12(3): 279-286

Onwukaeme, D. N, Ikuegbvweha, T. B and Asonye, C. C., (2007). Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of *Pycanthus Angolensis* Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits. *Tropical Journal of Pharmaceutical Research*; 6(2): 725-730

Oskay, M, Oskay, D and Kalyoncu, F., (2009). Activity of Some Plant Extracts against Multi-Drug Resistant Human Pathogens. *Iranian Journal of Pharmaceutical Research*; 8(4): 293-300

Pesewu, G. A, Cutler, R. R, and Humber, D. P., (2008). Antibacterial activity of plants used in traditional medicines of Ghana with particular reference to Methicillin-Resistant *Staphylococcus aureus* (MRSA). Journal of Ethnopharmacology; 116(1): 102-111.

Poole, K., (2001). Multidrug Efflux Pumps and Antimicrobial Resistance in Pseudomonas aeruginosa and Related Organisms. *Journal of Molecular Microbiology Biotechnology;* 3(2): 255-264

Qa'dan, F, Thewaini, A. J, Ali, D. A, Afifi, R, Elkhawad, A, and Matalka, K. Z., (2005). The Antimicrobial Activities of *Psidium guajava* and *Juglans regia* Leaf Extracts to Acne-Developing Organisms, *The American Journal of Chinese Medicine*; 33(2): 197–204

Qureshi, S, Rai, M. K, and Agrawal, S. C., (1997). In vitro evaluation of inhibitory nature of extracts of 18–plant species of Chhindwara against 3-keratinophilic fungi. *Hindustan Antibiotic Bulletin*; 39(1-4): 56-60.

Rajeshkumar, N.Y. and Kattan, R. (2000): *Phyllanthus amarus* extract administration increases the lifespan of rats with hepatocellular carcinoma. *Journal of Ethnopharmacology*; 73(1-2): 215-219

Rattanachaikunsopon, P and Phumkhachorn, P., (2010). Contents and antibacterial activity of flavonoids extracted from leaves of *Psidium guajava. Journal of Medicinal Plants Research*; 4(5): 393-396

Ruiza, J, Capitanoa, L, Nuñeza, L, Castro, D, Sierraa, J. M, Hathac, M, Borregob, J. J, and Vilaa, J., (1999). Mechanisms of resistance to ampicillin, chloramphenicol and quinolones in multiresistant *Salmonella typhimurium* strains isolated from fish. *Journal of Antimicrobial Chemotherapy;* 43 (5): 699-702

San Francisco, M. and Cooper-Driver, G., (1984). Anti-microbial activity of Phenolic acid in *Pteridium aquilinum*. *American Fern Journal*; 74(3): 84

Sanches, N. R, Cortez, D. A. G, Schiavini, M. S, Nakamura, C. V, and Filho, P. D., (2005). An Evaluation of Antibacterial Activities of *Psidium guajava* (L.). *Brazilian Archives of biology and Technology*; 48 (3): 429-436

Sawer, I. K, Berry, M. I and Ford, J. L. (2005). The killing effect of *cryptolepine* on *Staphylococcus aureus*. *Letters in Applied Microbiology*; 40(1): 24–29

Soetan, k. O, Oyekunle, M. A, Aiyelaagbe, O. O. and Fafunso, M. A., (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench. *African Journal of Biotechnology*; 5 (23): 2405-2407

Sofowora, A., (1982). Medical Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd. New York, pp 214

Sofowora, A., (1993). Medicinal plants and traditional medicine. Spectrum books Limited, Ibadan, Nigeria. pp. 224 -227

Sunita, A. C, Krupa, V. G, and Ankit, B. C., (2010). Comprehensive Review on World Herb Trade and Most Utilized Medicinal Plants. *International Journal of Applied Biology and Pharmaceutical Technology*; 1(2): 510-517

Tambekar, D. H, Dahikar, S. B, Lahare, M. D. (2009). Antbacterial potentials of some herbal preparations available in India. *Research journal of medicine and medical science*; 4(2): 224-227

Taylor, L., (2005). The healing power of rainforest herbs; A guide to understanding and using herbal medicinal. Square One Publishers, New York, USA. ISBN: 0-7570-0144-0

Tenover, F. C., (2006). Mechanisms of Antimicrobial Resistance in Bacteria. *The American Journal of Medicine*; 119(6A): S3-S10

Thong, K.L., Cheong, M.Y, Puthucheary, S, Koh, C.L, and Pang, T., (1994). Epidemiologic analysis of sporadic *Salmonella typhi* isolates and those from outbreaks by pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*; 32: 1135-1141.

Umeh, O. and Berkowitz, L. B., (2009). Klebsiella Infections. Contributor Information and Disclosures. http://emedicine.medscape.com/article/219907-overview (accessed 2011 January 12)

Wannissorn, B, Jarikasem, S, and Soontorntanasart, T., (1996). Antifungal activity of lemon grass and lemon grass oil cream. Phytotherapy Research; 10(7): 551-554

Wen-Chien, K, Paterson, D. L, Sagnimeni, A. J, Hansen, D. S, Gottberg, A. V, Mohapatra, S, Casellas,. J. M, Goossens, H, Mulazimoglu, L, Trenholme, G, Klugman K. P, McCormack, J, and Yu, V. L., (2002). Community-Acquired Klebsiella pneumoniae Bacteremia: Global Differences in Clinical Patterns. *Emerging Infectious Diseases*; 8(2): 160-166

White, N. J and Nosten, F., (1993). Advances in chemotherapy and prophylaxis of malaria. *Current Opinion in Infectious Diseases*; 6(4): 323-330

WHO, (2000). *The World Health Report. Health Systems: Improving Performance*. WHO, Geneva.

WHO, (2001). Legal Status of Traditional Medicine and Complementary/Alternative Medicine, A Worldwide Review, pp. 26

WHO, (2002). WHO Traditional Medicine Strategy 2000-2005, WHO Geneva. (*whqlibdoc.who.int/hq/2002/WHO_EDM_TRM_2002.1.pdf*)

WHO, (2003). Traditional Medicine – Report by the Secretariat. Fifty-Sixth World Health Assembly. Provisional agenda item 14.10. pp 1-4

WHO, (2005). National Policy on Traditional Medicine and Regulation of Herbal Medicine - Report of a WHO Global Survey. WHO, Geneva. pp 1-156

WHO, (2011). Antimicrobial resistance – Fact sheet N°194 http://www.who.int/mediacentre/factsheets/fs194/en/

Williams, L. A. D., (2006). Ethnomedicine Editorial. *West Indian Medicine Journal*; 55(4): 215

Wunderlin, R. P and Hansen, B. F., (2002). Atlas of Florida vascular plants online. http://www.plantatlas.usf.cdu/universityof south florida tampa. (accessed 2010 December 11)

Zhang, X., (2010). Regulatory Situation of Herbal Medicines A worldwide Review. *www.who.int/medicinedocs/pdf/whozip57e/whozip57e.pdf* (accessed 2010 December, 2010)

APPENDICES

APPENDIX 1

Solid Media

A MacConkey Agar

This is a differential medium for the differentiation and isolation of the *Enterobacteriaceae*. The medium also supports the growth of *Staphylococcus* and *Enterococci*, but inhibits the growth of *Streptococci* and *Haemophilus*. Swarming of *Proteus* is prevented because of the absence of Sodium Chloride which provides a low electrolyte medium which prevents most *Proteus spp* from spreading.

To prepare the medium, 48.5 grams of powder was dispensed in one litre of deionised water and allowed to soak for 10 minutes. It was then warmed to boil to dissolve completely after which it was sterilized by autoclaving for 15 minutes at 121º C. It was the poured into sterile petri dishes and allowed to solidify.

B Urea Agar

This medium is a composite one containing urea. Some microorganisms are able to hydrolyze urea by producing hydrolyzing enzymes while others are not. It is therefore for the differentiation and isolation of microorganisms that decompose urea.

To prepare the medium 2.4 grams of the base was suspended in 95mls of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 115º C for 15 minutes. Aseptically, 5mls of sterile 40% urea solution was added, and mixed well. Still in the liquid form, 5ml volume of the medium was aseptically distributed into sterile bijou bottles and allowed to set in the slope position. The pH of the ready use medium was approximately 6.8

C Simon's Citrate Agar

This is a synthetic medium for differentiation and isolation of the Enterobacteriaceae.

To prepare the medium 22 grams of the powder was added to one liter of distilled water and allowed to stand for 15 minutes. It was then heated to dissolve completely after which it was distributed into test tubes in 5ml volumes and sterilized in the autoclaved at 121º C for 15 minutes. The medium in the bottles was allowed to cool and solidify in the slant position.

D Triple Sugar Iron Agar (TSI)

TSI is a composite medium for the differentiation of the *Enterobacteriaceae* by three sugar fermentations and hydrogen sulphide production.

This medium was prepared by suspending 65 grams in one liter of distilled water and brought to boil to dissolve completely. After mixing well, the medium was distributed into capped test tubes. The tubes with media were sterilized by autoclaving at 121º C for 15 minutes after which they were allowed to set in sloped form with a butt about one inch long.

E Muller-Hinton agar (MHA)

MHA is used for Antibiotic sensitivity testing.

MHA medium was prepared by suspending 38 gram of the dehydrated powder in 1000ml of distilled water. It was then boiled to dissolve completely after which it was sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to cool in a 50º C water bath for about thirty-five minutes. The medium was poured aseptically into sterile glass plastic, flat-bottomed petri dishes (100 mm diameter) on a level, horizontal surface to give a uniform depth of approximately 4 mm and

allowed to cool to room temperature and, stored in a refrigerator 2-8º C. Plates were examined for sterility by incubating at 37º C for 18-24 hours.

F Nutrient Agar

Nutrient agar (NA) is a basal media for the isolation of nonfastidious organisms. The media is prepared by dissolving 20 gram of the dehydrated powder in 1000 ml of distilled water. It was then boiled to dissolve completely after which it was sterilized by autoclave at 121º C for 15 minutes and was allowed to cool in a 50º C water bath for about thirty-five minutes. The sterilized media was then poured into sterilized petri dishes and allowed to solidify then store in a refrigerator at a suitable temperature of 2-8º C. Plates were examined for sterility by incubating at 37º C for 18-24 hours.

APPENDIX 2

Liquid Media

A Peptone water

This is a medium rich in trypyophan. The medium was prepared by dissolving 15 grams of peptone (Oxoid L37) and Sodium Chloride 5.0 grams in one liter of distilled water. It was mixed well and distributed into capped test tubes and then sterilized by autoclaving at 121° C for 15 minutes and kept at room temperature for 29° C.

B Kovac's Reagent

This reagent is used for the detection of indole. It was prepared by dissolving 19 grams of 4-dimethylamino-benzaldehyde in 150 ml of iso-amyl alcohol. After dissolution 50 ml of concentrated hydrochloric acid was added to it. It was then stored in a refrigerator in an amber bottle.

APPENDIX 3

Control strains

Organisms	**Number**
Escherichia coli	ATCC 25922
Klebsiella pneumoniae	ATCC 33495
Pseudomonas aeroginosa	ATCC 27923
Staphylococcus aureus	ATCC 25923
Staphylococcus saprophyticus	ATCC 15305
Proteus mirabilis	ATCC 49565
Salmonella typhi	ATCC 19430
Salmonella typhimurium	ATCC 14028

APPENDIX 4

BIOCHEMICAL REACTION OF SOME ENTEROBACTERIA AND OTHER ENTERIC ORGANISMS.

						KIA MEDIUM			
SPECIES	**UREA**	**CIT**	**MOT**	**OX**	**IND**	**SLOPE**	**BUTT**	**H_2S**	**GAS**
Escherichia coli	-	-	+	-	+	Y	Y	-	+
Klebsiella pneumoniae	+	+	-	-	-	Y	Y	-	+
Proteus mirabilis	+	+	+	-	-	R	Y	+	+
Salmonella typhi	-	-	+	-	-	R	Y	+	-
Salmonella typhimurium	-	+	+	-		Y	R	+	+

KEY

UREA – Urease

CIT – Citrate test

MOT – Motility

IND – Indol test

OX – Oxidase test

Y – Yellow (acid reaction)

R – Red-pink (alkaline reaction)

H_2S – Hydrogen Sulphide

APPENDIX 5

Table 17: Toxicity Classes: Hodges and Sterner Scale

		Routes of Administration			
		Oral LD_{50}	**Inhalation LD_{50}**	**Dermal LD_{50}**	
Toxicity Rating	**Commonly used Term**	**(Single dose to rats mg/kg)**	**(exposure of rats for 4 hours) ppm**	**(single application to skin of rabbits mg/kg)**	**Probable Lethal Dose for Man**
1	Extremely Toxic	1 or less	10 or less	5 or less	1grain (a drop)
2	Highly Toxic	1 - 50	10 - 100	5 - 43	4ml (1 teaspoon)
3	Moderately Toxic	50 - 500	100 - 1000	44 - 340	30 ml (1 fluid ounce)
4	Slightly Toxic	500 - 5000	1000 – 10,000	350 - 2810	600 ml (1 pint)
5	Practically Non - toxic	5000 – 15,000	10,000 – 100,000	2820 – 22,590	1 liter (or 1 quart)
6	Relatively Harmless	15,000 or more	100,000	22,600 or more	1 liter (or 1 quart)

Source: Lancaster, 2010

APPENDIX 6

Table 18: MIC (mg/ml) of the ethanolic Antibact extract against pathogenic organisms

	MIC of plant extracts (mg/ml)											Control	
Test organisms	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**	**Chl**	**blank**
S. saprophyticus(1)	-	-	-	-	-	+	+	+	+	+	+	+	-
S. saprophyticus(2)	-	-	-	-	-	+	+	+	+	+	+	+	-
S. saprophyticus ATCC15305	-	-	-	-	-	+	+	+	+	+	+	+	-
P. mirabilis(1)	-	-	-	-	+	+	+	+	+	+	+	+	-
P. mirabilis(2)	-	-	-	-	-	+	+	+	+	+	+	+	-
P. mirabilis ATCC 49565	-	-	-	-	-	+	+	+	+	+	+	+	-
P. aeruginosa(1)	-	-	-	+	+	+	+	+	+	+	+	+	-
P. aeruginosa(2)	-	-	-	-	+	+	+	+	+	+	+	-	-
P. aeruginosa ATCC 27853	-	-	-	-	-	+	+	+	+	+	+	+	-
S. aureus(1)	-	-	-	+	+	+	+	+	+	+	+	+	-
S. aureus(2)	-	-	-	-	-	+	+	+	+	+	+	+	-
S. aureus ATCC 25923	-	-	-	-	-	-	+	+	+	+	+	+	-
E. coli (1)	-	-	-	-	+	+	+	+	+	+	+	+	-
E. coli (2)	-	-	-	-	+	+	+	+	+	+	+	+	-
E. coli ATCC 25922	-	-	-	-	-	+	+	+	+	+	+	+	-
S. typhi (1)	-	-	-	-	+	+	+	+	+	+	+	+	-
S. typhi (2)	-	-	-	+	+	+	+	+	+	+	+	+	-
S. typhi ATCC 19430	-	-	-	-	-	+	+	+	+	+	+	+	-

KEY

Chl = Chloramphenicol

- = No Growth

+ = Growth

Table 19: MIC (mg/ml) of the aqueous Antibact extract against pathogenic organisms

	MIC of plant extracts (mg/ml)											Control	
Test organisms	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**	**chl**	**blank**
S. saprophyticus(1)	-	-	+	+	+	+	+	+	+	+	+	+	-
S. saprophyticus(2)	-	-	+	+	+	+	+	+	+	+	+	+	-
S. saprophyticus ATCC 15305	-	-	-	-	-	+	+	+	+	+	+	-	-
P. mirabilis(1)	-	-	+	+	+	+	+	+	+	+	+	+	-
P. mirabilis(2)	-	-	+	+	+	+	+	+	+	+	+	+	-
P. mirabilis ATCC 49565	-	-	-	-	+	+	+	+	+	+	+	-	-
P. aeruginosa(1)	-	-	-	-	+	+	+	+	+	+	+	+	-
P. aeruginosa(2)	+	+	+	+	+	+	+	+	+	+	+	+	-
P. aeruginosa ATCC 27853	-	-	-	+	+	+	+	+	+	+	+	-	-
S. aureus(1)	-	-	+	+	+	+	+	+	+	+	+	+	-
S. aureus(2)	-	-	+	+	+	+	+	+	+	+	+	+	-
S. aureus ATCC 25923	-	-	+	+	+	+	+	+	+	+	+	-	-
E. coli (1)	-	-	-	-	+	+	+	+	+	+	+	+	-
E. coli (2)	+	+	+	+	+	+	+	+	+	+	+	+	-
E. coli ATCC 25922	-	-	-	-	-	-	-	+	+	+	+	-	-
S. typhi (1)	-	-	-	-	+	+	+	+	+	+	+	+	-
S. typhi (2)	+	+	+	+	+	+	+	+	+	+	+	+	-
S. typhi ATCC 19430	-	-	-	-	-	+	+	+	+	+	+	-	-

KEY

Chl = Chloramphenicol

- = No Growth

+ = Growth

Table 20: Antibiogram of Gram negative organisms

Test organism	Zone of inhibition (mm)											
	CHL	**CRX**	**GEN**	**COT**	**TET**	**AMP**	**CTX**	**AMK**	**CXM**	**CXC**	**ERY**	**PEN**
K. pneumoniae(1)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**
K. pneumoniae(2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**
K. pneumoniae ATCC 33495	**19**	**8**	**14**	**0**	**11**	**0**	**23**	**9**	**22**	**0**	**0**	**0**
P. mirabilis(1)	**10**	**20**	**14**	**0**	**8**	**0**	**29**	**16**	**29**	**0**	**0**	**0**
P. mirabilis(2)	**0**	**18**	**13**	**0**	**0**	**0**	**25**	**14**	**28**	**0**	**0**	**0**
P. mirabilis ATCC 49565	**14**	**19**	**9**	**0**	**0**	**0**	**25**	**10**	**28**	**0**	**0**	**0**
S. typhimurium(1)	**0**	**0**	**14**	**0**	**8**	**0**	**20**	**14**	**23**	**0**	**0**	**0**
S. typhimurium(2)	**0**	**0**	**0**	**19**	**8**	**0**	**20**	**13**	**25**	**0**	**0**	**0**
S. typhimurium ATCC 14028	**0**	**9**	**13**	**0**	**0**	**0**	**23**	**14**	**28**	**0**	**0**	**0**
P. aeruginosa(1)	**0**	**19**	**13**	**0**	**0**	**0**	**27**	**13**	**0**	**0**	**0**	**0**
P. aeruginosa(2)	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**19**	**26**	**0**	**0**	**0**
P. aeruginosa ATCC 27853	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**
E. coli (1)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
E. coli (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
E. coli ATCC 25922	**18**	**0**	**14**	**19**	**0**	**0**	**24**	**15**	**22**	**0**	**0**	**0**
S. typhi (1)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S. typhi (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S. typhi ATCC 19430	**14**	**20**	**18**	**20**	**0**	**0**	**30**	**18**	**34**	**0**	**0**	**0**

KEY:

AMK – Amikacin (30 µg/disc) **AMP** – Ampicillin (10 µg/disc)

PEN – Penicillin (10 i·u/disc) **CXM** – Cefixime (30 µg/disc)

CXC – Cloxacillin (5 µg/disc) **COT** – Cotrimoxazole (25 µg/disc)

CRX – Cefuroxime (30 µg/disc) **TET –** Tetracycline (30 µg/disc)

ERY – Erythromycin (15 µg/disc) **CHL –** Chloramphenicol (30 µg/disc)

GEN – Gentamicin (10 µg/disc) **CTX** – Cefotaxime (30 µg/disc)

Table 21: Antibiogram of Gram positive organisms

	Zone of inhibition (mm)											
Test organism	**ERY**	**TET**	**AMK**	**AMP**	**CRX**	**CTX**	**COT**	**GEN**	**CHL**	**CXM**	**CXC**	**PEN**
S. aureus(1)	**0**	**8**	**14**	**0**	**19**	**25**	**0**	**16**	**0**	**30**	**0**	**0**
S. aureus(2)	**0**	**0**	**0**	**0**	**19**	**26**	**0**	**15**	**0**	**28**	**0**	**0**
S. aureus ATCC 25923	**0**	**13**	**16**	**0**	**15**	**0**	**18**	**17**	**21**	**0**	**0**	**18**
S. saprophyticus(1)	**0**	**14**	**20**	**10**	**0**	**0**	**0**	**19**	**0**	**0**	**0**	**0**
S. saprophyticus(2)	**0**	**8**	**15**	**11**	**14**	**9**	**0**	**16**	**0**	**0**	**0**	**0**
S. saprophyticus ATCC 15305	**0**	**10**	**12**	**0**	**12**	**10**	**0**	**18**	**14**	**17**	**0**	**20**

KEY:

AMK – Amikacin (30 µg/disc)

AMP – Ampicillin (10 µg/disc)

PEN – Penicillin (10 i·u/disc)

CXM – Cefixime (30 µg/disc)

CXC – Cloxacillin (5 µg/disc)

COT – Cotrimoxazole (25 µg/disc)

CRX – Cefuroxime (30 µg/disc)

TET – Tetracycline (30 µg/disc)

ERY – Erythromycin (15 µg/disc)

CHL – Chloramphenicol (30 µg/disc)

GEN – Gentamicin (10 µg/disc)

CTX – Cefotaxime (30 µg/disc)

Table 22: Phytochemical components of the herbal products

	AQUEOUS EXTRACT				ETHANOL EXTRACT			
	P. fraternus	***H. opposita***	***C. citratus***	***P. guajava***	***P. fraternus***	***H. opposita***	***C. citratus***	***P. guajava***
Saponins	-	+	+	+	-	-	+	+
Reducing sugar	+	+	+	+	+	+	+	+
Cyanogenic glycoside	-	-	-	-	-	-	-	-
Phenolics	+	+	+	+	+	+	+	+
Polyuronides	+	+	-	+	-	-	+	+
Alkaloids	-	-	-	-	+	-	-	-
Anthracenosides	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	+	-
Phytosterols	-	+	-	+	-	-	-	+
Triterpenes	+	-	+	+	+	+	+	+

KEY

- **Negative**
+ **Positive**