KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

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Antimycobacterial Activities of Selected Medicinal Plants and Formulations in the Management of *Mycobacterium tuberculosis* and *Mycobacterium bovis*

By

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JUNE, 2016

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By

Richard Ankomah (Bed Science)

A Thesis submitted to the Department of Theoretical Biology, Faculty of Biosciences, College of Science, in Partial Fulfilment of the requirements for the Master of Philosophy Degree in MICROBIOLOGY

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DECLARATION

The experimental work described in this thesis was conducted in the Bacteriology Department of the Nougchi Memorial Institute for Medical Research, University of Ghana, Legon, the Bacteriology Laboratory of the Kumasi Center for Collaborative Research (KCCR) in Tropical Medicine, and the Faculty of Pharmacy and Pharmaceutical Science, Kwame Nkrumah University of Science and Technology (KNUST) from August, 2011 to November, 2015, under the supervision of Dr. F. C. Mills-Robertson and Mrs. Linda A. Ofori. This study is the result of my own investigations and has not been submitted in any other form to another University.

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ABSTRACT

Recent years have witnessed an increase in the use and search for new drugs derived from plants. Microbiologists, Ethnopharmacologists, Botanists, Natural-product chemists and other related bodies are all trying to discover phytochemicals that could be developed for the treatment of infectious diseases. Tuberculosis is an endemic and pandemic bacterial disease caused by the *Mycobacterium tuberculosis* complex. Treatment, prevention and the rate at which tuberculosis is spreading has been the concern of World Health Organization and individuals. Isoniazid, Ramfipicin, and Ethambutol, among others have been the drugs of choice for the treatment of tuberculosis; however, these drugs take longer periods of time before their positive effects are noticed and experienced and may even produce adverse side effects on the human system. In this study, nine medicinal plants were selected through ethno-botanical survey in Southern part of Ghana. These medicinal plants used to treat respiratory diseases, stomach ailments and other microbial infections were evaluated for their anti-tubercular activity. The nine selected plant species were tested individually and as part of formulations against drug sensitive strain of *Mycobacterium tuberculosis* (H37Rv) and *Mycobacterium bovis* at concentrations ranging from 1.0 to 5.0 mg/ml using Lowenstein-Jensen egg medium at bacteriology department of KCCR and Nougchi Memorial Institute for Medical Research. Phytoconstituents observed were terpenoids, phenols, tannins, flavonoids, steroids, saponins, glycosides, and alkaloids. *Allium sativum* inhibited the growth of both *Mycobacterium tuberculosis* (H37Rv) and *Mycobacterium bovis* at concentrations of 5.0 mg/ml, 2.5 mg/ml and 1.0 mg/ml with other individual plants inhibiting the test organisms at 5.0 mg/ml. Formulations from a combination of *Allium sativum* and *Lantana hispida* formulation also inhibited the growth of both strains at the lowest concentration of 1.0mg/ml with other formulations inhibiting to varying degrees. Thus, *Allium sativum* and a formulation from a combination of *Allium sativum* and *Lantana hispida* exhibited great potential in inhibiting the growth of *Mycobacterium tuberculosis* and *Mycobacterium bovis*; hence could be used to manage infections caused by these microbes. The study has also scientifically substantiate the used of selected medicinal plants used in the treatment of tuberculosis in Ghana and also revealed scientifically that, there is high potential of medicinal plants which can treat tuberculosis even better than the standard drugs.

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DEDICATION

I would like to dedicate this piece of work to the following individuals who mean a lot to me in my life:

- My wonderful and precious sons; Richard Ankomah Junior and Cletus Damian Ankomah
- My understandable and lovely wife, Mrs. Grace Boadiwaa Ankomah, and
- My hard-working Supervisors.

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CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND INFORMATION TO THE STUDY

Microbial diseases have been with man long before civilization and since the realization of this canker, man has not relented in his quest to eliminate, reduce, cure or treat such infections. It is, however, very surprising that even with the emergence of scientific knowledge and discoveries, the issue of microbial diseases has not become a thing of the past. Man has, therefore, suffered a great deal from microbial diseases and continues to suffer from them. Antibiotics have been relied on to provide relief but antibiotic resistance has become a global concern as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Parekh & Chanda, 2007) such as the *Mycobacterium tuberculosis* complex.

Tuberculosis is a severe infectious disease that is caused by species of the *Mycobacterium tuberculosis* complex with *Mycobacterium tuberculosis*, *Mycobacterium africanum* and *Mycobacterium bovis* being human pathogens. *Mycobacterium microtti* infect voles, guinea-pigs, rabbits and sometimes bovines (Wayne, 1984; Cousins *et al.,* 2003) while *Mycobacterium pinnipideii* is responsible for tuberculosis in seals (Cousins *et al.,* 2003). *Mycobacterium bovis* is, in addition, responsible for pulmonary disease in bovine and sometimes to mammary lesions with passage of tubercle bacilli in milk; however, infections which in the past were often transmitted through the oral route in drinking milk from infected cows, are uncommon in most countries today (Porter & McAdam, 1994). *Mycobacterium africanum* is responsible for 20 to 80 % of human tuberculosis in sub-Saharan Africa, and also for some tuberculosis cases diagnosed outside the

African continent. Virtually, all new infections with *Mycobacterium tuberculosis* are acquired via airborne transmission and the sources of infections are persons with tuberculosis of the lungs and larynx who are coughing. The coughing produces tiny infectious droplets, 1-5µm in size, known as droplet nuclei. In indoor environments, these droplet nuclei can remain suspended in the air for long period of time unless they are removed by ventilation, filtration or ultraviolet irradiation. Certain domestic animals come in contact with people having tuberculosis infections, and these people also can be infected and become a source of infection (Grange, 1990; Cousins *et al.*, 2003). Tuberculosis (TB) is principally a disease of poverty, with 95 per cent of cases and 98 per cent of deaths occurring in developing countries (Sharma & Mohan, 2004). Each year, an estimated eight million new cases and two million deaths occur due to TB worldwide (Kishore *et al.*, 2007). In 1993, the World Health Organization (WHO) took an unprecedented step and declared TB to be a global emergency (Sharma *et al.*, 2004). The exact cause for the formation of the global emergency is unknown, although it is thought that it could be because of the resurgence of TB due to HIV infection as well as Multiple Drug Resistant Tuberculosis (MDR-TB) resulting from inefficient management.

WHO (2000) estimated that between the year 2000 and 2020, close to one billion people will die from tuberculosis and that majority of the infected persons who form majority of world's population reside in developing countries (Snider, Raviglone & Kochi, 2005). In a report by WHO (2012), 9.4 million tuberculosis cases were reported worldwide in which 3.6 million were females among whom 500,000 patients presented co-infection with the Human Immunodeficiency Virus (HIV). There were also 1.8 million deaths, with an average of 4,500 deaths daily, due to the disease. In 2011, there were an estimated 8.7 million incident cases of

TB (range, 8.3 million–9.0 million) globally, equivalent to 125 cases per 100 000 population (WHO Global Report on Tuberculosis, 2012). The rise in the number of tuberculosis incidence has been attributed to Human Immunodeficiency Virus/ Acquired immune deficiency syndrome infection and also to the appearance and development of tuberculosis resistant drugs, both multidrug resistant and extremely drug resistant strains (WHO, 2009). Drug-resistant tuberculosis may be caused by non-compliance with the time for treatment, prescription of an inadequate treatment regimen, and administration of insufficient drugs, among others. Treatment of Multidrug-Resistant tuberculosis needs the use of minimum of three antibiotics. Extremely drug-resistant tuberculosis, on the other hand, is resistant to the second-line-drugs in addition to being resistant to the first-line drugs; hence, making the management of these infected people very difficult. Thus, the success of curing cases of Multidrug Resistance tuberculosis and Extremely Drug-Resistant tuberculosis is very low (Barstian & Colebunders, 2000).

Before the onset of modern science, ancient people relied on what may be referred to as "traditional or indigenous science" to treat some microbial diseases. Even though infections were not diagnosed based on scientific techniques, treatments were appreciably effective. Fortunately, most of the potent orthodox drugs available today may have their bases from either a whole plant or a component of the plant (Iwu, Duncan & Okunji, 1999).

In view of the deadly and highly infectious nature of microbes and their activities, coupled with the expensive and associated side effects of orthodox drugs, the need for an alternative, especially medicinal plants with antimicrobial properties is highly recommended for extensive studies. In Ghana, plants have been investigated as sources of antimicrobials and bioactive agents for the treatment of human disease over several years, however, only a few of their endemic plants have been used to treat diseases such as tuberculosis even though it has diverse and unique flora.

1.2. OBJECTIVES *OF THE STUDY*

The main objective of this study was to investigate, *in vitro*, the anti-TB activities of selected Ghanaian medicinal plants and formulations in the management of *Mycobacterium tuberculosis* and *Mycobacterium bovis*.

The specific objectives of this study were to:

(i) determine the susceptibility of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* to extracts from the selected individual medicinal plants.

(ii) determine the susceptibility of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* to formulations made from some of the individual medicinal plants.

(iii) determine the phytoconstituents of the selected medicinal plants as well as formulations that showed successful activity.

1.3. JUSTIFICATION

Inability to control *Mycobacterium tuberculosis* and *Mycobacterium bovis* has attracted and continues to attract the concern of Government, Microbiologist, Researchers, Health organizations and the entire public nationwide. In this regard, it is hoped that the findings of the present study will provide all stakeholders information on some of the factors which significantly contribute to the knowledge of the activities of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Infectious diseases such as tuberculosis and HIV cases are quite prevalent in Ghana, particularly in rural area, where an astounding number and variety of plants are used by the indigenous people

to treat these diseases without any scientifically determined information. In this study, anti-tuberculosis activities of medicinal plants obtained in Ghana were examined. The evaluation of these plants for biological activity is necessary, and also as a possible lead for new drugs or herbal preparations. This study provided valuable information for alternative treatment for tuberculosis and also isolation of bioactive compounds from the studied plant species.

The study revealed that plant extracts have control of the growth and also showed resistance to *Mycobacterium tuberculosis* and *Mycobacterium bovis.* It also helped in identifying some factors and behaviours, which contribute to the susceptibility level of *Mycobacterium tuberculosis* and *Mycobacterium bovis* so that stakeholders can adopt strategies to control tuberculosis.

1.4. RESEARCH HYPOTHESIS

Hi – There is significant effect of medicinal plant extracts on the susceptibility of *Mycobacterium tuberculosis and Mycobacterium bovis (BCG).*

Ho – There is no significant effect of medicinal plant extracts on the susceptibility of *Mycobacterium tuberculosis* and *Mycobacterium bovis (BCG).*

Hi – There is significant effect of formulation of medicinal plant extracts on the susceptibility of *Mycobacterium tuberculosis* and *Mycobacterium bovis (BCG).*

Ho – There is no significant effects of formulation of medicinal plant extracts on the susceptibility of *Mycobacterium tuberculosis* and *Mycobacterium bovis (BCG).*

Hi – There is significant effect of medicinal plant extracts on the susceptibility of *Mycobacterium bovis (BCG).*

Ho – There is no significant effect of medicinal plant extracts on the susceptibility of *Mycobacterium bovis (BCG).*

1.5. RESEARCH QUESTIONS

- What is the level of susceptibility of *Mycobacterium tuberculosis* and *Mycobacterium bovis* (BCG) to the combination of medicinal plant extracts as compared to other standard drugs in Ghana?
- What are the effects of the extracts of the individual selected medicinal plants and their formulations in controlling *Mycobacterium tuberculosis* and *Mycobacterium bovis (BCG)*?

1.6 SIGNIFICANCE OF THE STUDY

The problem of curing tuberculosis in the shortest possible time has been the concern of all and the most important thing is to determine an alternative medicine to solve that phenomenon. This research would therefore be beneficial to all stakeholders with an alternative to the treatment of tuberculosis infections currently with six months treatment on the cocktail of various antibiotics.

Secondly, all the stakeholders would be informed on the importance of screening and identifying the bioactive component of the medicinal plants in Ghana for treatment of tuberculosis. Moreover, pharmaceutical researchers would be inspired to analyze the various plants in the forest of Ghana to come out with alternative medicines for the treatment of tuberculosis in the shortest possible time.

Lastly, this study hopes to scientifically substantiate the local use of selected medicinal plants in the treatment of tuberculosis in Ghana.

1.7 LIMITATION OF THE STUDY

Mycobacterium tuberculosis H37Rv and *Mycobacterium bovis* are not the only causative organisms human tuberculosis but there are other strains such as *Mycobacterium africanum* However, due to lack of sponsorship and financial difficulties to undertake such research, I could not dive into all the strains as well as other causative organisms of tuberculosis in humans to find out their susceptibility to the extracts of the medicinal plants.

Again, I faced with the problem of travelling from Abetifi to Accra in the Greater Accra Region and Kumasi also in the Ashanti Region several times for extraction, culturing, reading and reporting and also to see my supervisors for discussion and guidelines.

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CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. TUBERCULOSIS

TB is a disease known since time in memory, and dates back to around 8000 BC (Ayyazian, 1993; Basel, 1998). The first evidence of tuberculosis among mankind was observed in the decay remains of the skeleton and muscular remains of the Egyptian mummies in 2400 BC (Haas, 1996); however, its causative organism was not tested to know the actual causative organism of the disease as either *Mycobacterium bovis* or *Mycobacterium tuberculosis*. In 17th and 18th centuries, Tuberculosis outbreak increased in other continents and was recorded as the largest cause of death in America and Western Europe. After century of the outbreak of TB in the Western Europe and

the United States, tuberculosis then spread in rapidly to Africa, Eastern Europe, South America and Asia (Bloom & Murray, 1992).

In recent years, Tuberculosis is directly next to Human Immunodeficiency Virus/ Acquired immune deficiency syndrome causing death throughout the continent from a single human disease causing organism, killing millions of peoples. It is second to Human Immunodeficiency Virus/ Acquired immune deficiency syndrome (HIV/AIDS) (WHO, 2012), and claiming more lives than most of the tropical illnesses put together (Zumla & Grange, 1998). In recent years there has been a dramatic resurgence of tuberculosis in people also infected with HIV that have caused marked increase in tuberculosis in some countries (WHO, 2012). Since it has the ability to destroy the immune system, Human Immunodeficiency Virus, Acquired immune deficiency syndrome has stoodout as the important influential increment of latent tuberculosis infection to clinical diseases (Selwyn, Hartel & Lewis, 1989). In 2007, there were global cases of tuberculosis which affected almost 9.27 million people, an increase from 9.24 million cases in 2006, 8.3 million cases in 2000 and 6.6 million cases in 1990 (WHO, 2009). The majority of the estimated number of cases in 2007 was in Asia (55%) and Africa (31%), with small proportions of cases in the Eastern Mediterranean Region (6%), the European Region (5%) and the Region of the Americas (3%). India, China, Indonesia, Nigeria, and South Africa were the 5 countries that ranked first to fifth in terms of the total numbers of cases in 2007 estimated figures: 2.0 million,

1.3 million, 0.53 million, 0.46 million, and 0.46 million tuberculosis cases respectively. From the 9.27 million tuberculosis cases in 2007, an estimated 1.37 million (14.78%) were HIV-positive, 78% of the HIV-positive cases were detected in Africa with 12% being in the Southern Asia to Eastern Asia. Although the total number of tuberculosis infection is increasing in absolute terms due to population growth, the number of cases per capital is falling. The rate of decline is slow,

thus less than 1% per year. Globally, rates peaked at 142 cases per 100 000 population in 2004. In 2007, there were an estimated 139 incident cases per 100 000 population. The Incident rates are falling in five of the six World Health Organisation regions (WHO, 2009). The effect of Human Immunodeficiency Virus/ Acquired immune deficiency syndrome (HIV/AIDS) infection on the tuberculosis has placed the greatest impact on those countries where the occurrence of tuberculosis infection is very high in young adults (Bloom, 1994).

2.2. DESCRIPTION OF MYCOBACTERIUM SPECIES

The genus *Mycobacterium* (order: Actinomycetales, family: Mycobacteriaceae) is made up of about 50 acid-fast, aerobic, non-motile and non-spore-forming bacterial species. Majority of these species are environmental saprophytes, existing in various substrates such as plants, soil, water, bird and mammals. The genus is divided into the fast-growers and the slow-growers. The fast-growers are commonly not disease causing organism whiles few can cause infections by chance or due to circumstances among human and animal activities (Grange & Yates, 1986). The disease-causing organisms are the slow-growers which cause tuberculosis in animals and peoples (McGaw, Lall, Meyer & Eloff, 2008). *Mycobacterium africanum* is a member of the Mycobacterium tuberculosis complex, comprising of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium canettii*. *Mycobacterium africanum, Mycobacterium tuberculosis* and *Mycobacterium bovis* have been confirmed by their deoxyribonucleic acid to deoxyribonucleic acid hybridization to related closely, multi-locus electrophoresis of enzyme, and sequencing of the 16S ribosomal Ribonucleic Acid gene to ribosomal Deoxyribonucleic Acid and the 16S to 23S ribosomal

Deoxyribonucleic Acid internal transcribed spacer region (Feizabadi, Robertson, Cousins, & Hampson, 1996; Frothingham, Hills, & Wilson, 1994; Kirschner, Springer, Vogel, Meier, Wrede Bange & Bottger, 1993; Niemann, Harmsen, Rush-Gerdes & Richter, 2000; Wayne & Kubica, 1986). In spite of the high level of closeness at the deoxyribonucleic acid level of the species, the various species of *Mycobacterium tuberculosis* complex distinct from the patients' location prevalences and pathogenicity. For this reason, the exact species differentiation of clinical strains remain important for epidemiological and public health purposes, since it was first described in 1968 (Castets, *et al.,* 1968). *Mycobacterium africanum* has been identified in several parts of Africa and it contributes to almost 65% of clinical isolates from individuals with pulmonary tuberculosis (Niemann, *et al.,* 2002; Niobe-Eyangoh, *et al.,* 2003; Schwander, *et al.,* 1995; Vianna-Niero, *et al.,* 2001). Current surveys have shown an increase in the outbreak and spread of *Mycobacterium africanum* in other parts of Africa. For instance, close to 5% of individuals suffering from tuberculosis in Ivory Coast, almost 10% individuals suffering from tuberculosis in Cameroon (Niobe-Eyangoh *et al.,* 2003), and close to 60% of individuals suffering from tuberculosis from Guinea-Bissau (Bonard *et al.,* 2000; Kallenius *et al.,* 1999) were detected have been infected from *Mycobacterium africanum*.

Based on the biochemical properties, two main *Mycobacterium africanum* subgroups have been identified, and the identified subgroups relate to their geographic origins, that is subtype I (cluster G) in West Africa and subtype II (cluster F) in East Africa (Niemann *et al.,* 2002). The analysis of biochemical properties revealed that *Mycobacterium africanum* subtype I is closely similar to *Mycobacterium bovis*, whereas subtype II looks more like *Mycobacterium tuberculosis* (Niemann *et al.,* 2002).

2.2.1. Mycobacterium tuberculosis

Mycobacterium tuberculosis was documented on the 24th March 1882 by Robert Koch. He was awarded with Novel Prize in medicine and physiology in 1905 (Newton *et al*., 2000).

Mycobacterium tuberculosis, also referred to as Koch's bacillus, belongs to the tuberculosis complex and its related mycobacterial causative organisms include *Mycobacterium bovis*, *Mycobacterium microti* and *Mycobacterium africanum* (causative tuberculosis agent in West Africa), *Mycobacterium sacrefulaceum* (causing other infections in patients with AIDS), *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium lepraemurium* (which cause tuberculosis in rats and cats), and *Mycobacterium laprae* (cause leprosy in man) (Horne, 1996). The purpose of core factor in the pathogenesis of tuberculosis has still not been identified; but it is believed to be significant due to the fact that it prevents and stimulate secretion of tumour necrosis factor alpha by macrophages (Brennan, 1998).

Mycobacterium tuberculosis does not show the biochemical characteristics of Gram-negative or Gram-positive bacteria and due that, *Mycobacterium tuberculosis* is not considered as either Gram-negative or Gram-positive bacteria (Camus *et al*., 2002). When a Gram stain is applied on *Mycobacterium tuberculosis*, its staining is extremely poor. *Mycobacterium* species, together with members of the genus *Norcadia*, are considered as acid-fast because they are not permeable to certain dyes and stains. Carbon-fuchsin and decolouring with acid alcohol is one of the staining methods for acid-fast *Mycobacterium tuberculosis*. After the use of Carbon-fuchsin and decolouring the smear with acid alcohol, methylene blue on different media such as LowensteinJensen is used to counterstain the smear. (Fadda & Roe, 1984).

Mycobacterium tuberculosis is a slow-grower bacillus that is spread mainly through the airways, and the disease is spread by inhalation of small droplets (1–10 microns) having only a few live *Mycobacterium tuberculosis* in it. The first target of *Mycobacterium tuberculosis* is normally the lower part of the lung. The tubercle bacilli are always taken up by lung macrophages and do survive and grow, forming the main target of *Mycobacterium tuberculosis*. Then the bacilli move to the lymphatic tissue with the help of the blood and lymphatic tissue throughout the body. The disease usually become silent without showing any positive sign clinically or at times the patient develop mild fever when the bacilli is sited at the local lymphatic system and often the immunity develops in a few weeks' time then infected person can test positive to TB (Girling, 1989). A common property of *Mycobacterium tuberculosis* disease is that the bacteria breeds intracellularly, normally in macrophages and through that the bacteria evade most of the patients defence pathway (Banki, Jenei, & Richards, 2000; Velasco-Velazquez, Berrera, GonzalezArenaz & Agramonte-Hevia, 2003).

During the latter part of 1980's, tuberculosis started spreading throughout the world, and now it kills over 2 million people every year globally. It is believed that 2 million people have come in contact with the TB bacilli and there is high danger of acquiring the tuberculosis disease (Gutierrez-Lugo *et al*., 2005). Most cases of deaths and death rate are record in the Africa. The two important influence factors for the fast transmission of tuberculosis in Africa are; overcrowded states of people that promote airborne spreading also peoples or humans with little natural resistance. Tuberculosis among humans can be considered as a result of three clearly different factors, namely the infection of a person in the community with *Mycobacterium tuberculosis* within a given period of time, the rapid growth of the disease immediately after such infection,

and rapid long spreading of the disease after its first infection, due to the regaining of latent bacilli (Raviglone *et al*., 1995; Bloom, 1994).

Mycobacterium tuberculosis affects closely 32% of the population worldwide. Each year close to 8 million of infected people contract tuberculosis, resulting in 2 million people dying as a result of the infection (WHO, 2006).

2.2.2. Transmission of tuberculosis

The main route of acquiring tuberculosis is through breathing in the polluted air containing the pathogen of the disease (Rom & Garay, 1996). Droplets nuclei begin to settle gradually whiles the remaining suspend in the atmosphere for long periods. A droplets nucleus are only obtained when the patient experiencing active pulmonary tuberculosis sings, coughs, sneezes or speaks.

Talking for 5 minutes can release about 3000 infectious droplet nuclei which is the same as coughing; sneezing can also release more than million particles of diameter fewer than 100 mm (Bloom, 1994). When breathed in, droplet nuclei normally go into the airways till they get to the alveoli. Bigger particles which might be exposed along the way are gotten raid off through the body's normal way of mechanism of air path clearance (Dannenberg, 1989). The first appearance of *Mycobacterium tuberculosis* infection in the body demands regulation by the body's immune system. After few days of infection, the patient's body has almost no immune defence against the infection by the tuberculosis pathogen. A little breathing in with no harmful effect multiplies freely within alveolar macrophages. Unrestrained bacterial multiplication continues until the development of tissue hypersensitivity and cellular immunity. *Mycobacterium tuberculosis* gets

stuck to the alveolar space through multiple complements which can be destroyed in the phagosome (Beyers, 1999).

2.2.3. Infection and Symptoms of Tuberculosis

When the pathogen of tuberculosis is found in the body but it does not always make the individual fall sick, it is described as TB infection. This is due to the fact that the healthy strong immune system which is not able to destroy the pathogen by itself, but it can keep the pathogen trapped in the alveoli of the lungs and inhibits its transmission from one person to the other. Since the pathogen is potent and has a thick coat cover to protect itself, it can stay dormant in the system for a lot of years. Individuals with tuberculosis infection having immune systems already weakened and having other types of lung ailment are most likely to suffer from tuberculosis (WHO, 2007). Symptoms of tuberculosis vary depending on the particular part of the body which the pathogens are multiplying. Common symptoms with the tuberculosis disease include chest pains, sputum with blood, weight loss, dry coughing which last for more than 2 weeks, fever, chills and sweating at night. Some people do feel joint pain just like arthritis at times when the tuberculosis pathogen stays in the bones. When not treated, a TB patient will infect 10 to 15 people averagely with tuberculosis yearly (Davies, 2003).

2.2.4. Tuberculosis prevention

The end result of Mycobacterium infection will be based on the patients' immune system. With many people infected with TB pathogen induces an immune response which is enough in order to

protect the system against the progress of the active illness (Bapela, 2005). Bacilli CalmetteGuerin (BCG) vaccine produces smallest infection and does not inflict high risk ailment (Young & Duncan, 1995). BCG vaccine was first used in 1921 to protect humans against TB; this BCG is a strain of *Mycobacterium bovis* attenuated through years of serial passage in culture. (Young & Duncan, 1995). Throughout the world, Bacilli Calmette-Guerin vaccines have been given to 100 million infants in recent years. These vaccines differ in cultural properties and its potential to induce sensitization to tuberculin but were derived from the original strain. There are different ways for vaccine administrations as well as differences in methods and techniques of producing the vaccine (Young, 1994).

2.2.5. Treatment of tuberculosis

Almost half of the individuals with pulmonary TB use to die at least 2 years after infection and only few were cure before the discovery of strong TB drugs were made available (Bartmann, 1988). With the introduction of effective anti-TB drug, patients put on bed rest and lengthy separation of patients with TB was seen as not important, and in theory at least, effective management was a reasonable aim for all individuals with TB (Bartmann, 1988). *Mycobacterium* has the natural ability to withstand most common chemotherapy agents and antibiotics. This might be due to the pathogen's highly hydrophilic cell envelope acting as a strong permeable barrier. The number of tuberculosis transmission and the killing of individuals, especially in developed countries has reduced greatly because of the discovery and introduction of a strong anti-mycobacterial agent's ethambutol (EMB) in the twentieth century and also decrease in poverty (Chan and Iseman, 2002). The discovery of Multi Drug Resistant Tuberculosis (MDRTB) in 1980 saw the number of TB infection worldwide increasing rapidly (Chan & Iseman, 2002).

The Multi Drug Resistance is defined as the form of resistance to more than two existing tuberculosis drugs, and are often dangerous, expensive and not simple to cure (Basso & Blanchard, 1998; Bastian & Colebunders, 2000). The association of TB with HIV in sub-Saharan Africa and many developing countries has recently complicated the case of MDR-TB (Corbett *et al*., 2003; Lurie *et al*., 2004; WHO, 2012). The TB case has also been worsened as a result of high emerging drug resistant tuberculosis (Core Curriculum on Tuberculosis, 2000). The effective chemotherapy of TB patients takes a longer period of half a year to 9 or more months with the first line tuberculosis drugs (Gautam *et al*., 2007). With the acquired drug resistant TB, capreomycin, cycloserine, kanamycin and ethionamide which are described as second-line drugs can be used, but they have serious and important side effects with close to 50% management rate (Gautam *et al*., 2007; Heym & Cole, 1997). The recent treatment decreases the pulmonary bacterial burden yet the curing time of half a year for non-immune suppressed patients and up to third quarter of the year for individuals having their immune system being suppressed which needed dependable curing efficacy (Quenelle *et al*., 2001; Bapela, 2005). The occurrence of drug resistant *Mycobacterium tuberculosis* has been of great worry now. Drug-resistance occurs when the first line drug is given alone and there are large numbers of active *Mycobacterium* population in the lesions. Drug resistance is largely believed to be the result of increased growth of sensitive organisms from mutant resistant bacilli located in wild strains before it come in contact with the drugs concerned (Mitchison, 1984). For the past 30 years, there have been no new discoveries of drugs to destroy or kill the TB pathogen (Gautam *et al*., 2007).. Therefore, there is an immediate and important need to search and come out with new affordable and effective TB drugs to kill pathogens as suggested by Gautam *et al*., (2007).

2.2.6. Targets of First-line Tuberculosis drugs.

Recent chemical treatment against tuberculosis mostly depends on drugs that prevent the pathogen's metabolism from functioning properly with a large emphasis on those that prevent the cell wall synthesis (Zhang, 2005). Due to the path of action of first and second line drugs, INH, EMB, ethionamide and cycloserine may be considered as cell wall inhibitors while RIF and quinolones are considered as nucleic acid producing inhibitors , STR and kanamycin are grouped as protein synthesis stoppers (inhibitors), and PZA prevent the membrane energy metabolism (Mitchison, 1980). Present tuberculosis drugs have the ability to focus on potent growing bacteria through prevention of cell activities. This means that the present chemotherapy is noted by an efficient bacterial activity but an extremely weak sterilising property which is said to possess ability to kill the slowly growing and metabolising bacteria that survive after most of the growing bacteria have been killed by bactericidal drug. Sterilising property also talks about the potential to get rid of latent bacteria that persisted inside the host macrophages (Mitchison, 1980).

2.3. TRADITIONAL MEDICINE

2.4. HERBAL MEDICINE

Herbalism is another name used for calling herbal medicine at times and the use of these plants for their medicinal purpose were used in all part of the world in the olden days (Duke, 2002). Traditional herbal users make use of the various plants parts to prevent, reduce and manage illnesses (Wijesekera, 1991; Criagg, *et al*., 2001). These plants and their respective parts release various chemical substances that work on the body. In recent years, the awareness on the

importance of medicinal plants has increased. Medicines from plants are available, less costly, safe and efficient to use and does not have any side effects. Plants selected for medicinal use over thousands of years constitute the most obvious choice of currently searching for therapeutically effective new drugs such as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson, *et al.*, 1996) and antihepatotoxic compounds(Balick, 1990). In the United States, about 25% of prescription drugs contain, at least, one active ingredient derived from plant materials, some are made from plants extracts, and others are synthesized to mimic a natural plant compound (Balick, 1990). Following the industrial revolution, a second generation of plant drugs have emerged based on scientific processing of plant extracts to isolate "their active constituents". Plants have been a good source of anti-infective agents for so many years. For instance isoquinoline alkaloids, emetine, obtained from the underground part of *Cephaelisi pecuanha,* and related species, have been used for many years as an amoebicidal drug for the treatment of abscesses due to the spread of *Entamoeba histolytic* infections. Investigators from various fields are, thus, researching into plants with the view of discovering valuable phytoconstituent with laboratories throughout the continent having found a lots of phytochemicals which have inhibitory effects on all types of microorganism *in vitro* (Cowan, 1999).

Herbal medicine has an old record in curing most of the illnesses (Holm *et al*., 1998). The use of herbal medicine for curing all forms of diseases has been practiced by humans for a very long time, and it is still currently being practised (WHO, 2014). Over the years, researchers have established a store of experimental information about the therapeutic values of local plants before orthodox medicinal practice surfaced. Through the period of trial and error, those herbalists and their apprentices have brought about a lot of knowledge on medicinal plants. From Iwu *et al*. (1999), the first development of medicine from plants was normally basic botanical that was employed

more or less in their crude form. Several effective medicines used in their natural state were thus selected as therapeutic agents based on empirical study of their application by traditional societies from different parts of world

According to WHO (2012), medicinal plants can be the best source of developing several drugs. Almost 80% of individual plants from developing countries are used as traditional medicines, which contain compounds derived from medicinal plants. Thus, plants need to be investigated thoroughly to better understand their properties, safety and efficiency (Arunkumar, *et al.*, 2009). Medicinal plants contain chemicals that provide definite physiological action on the human system. These bioactive compounds generally include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Mann, 1978; Edoga, 2005), which are synthesized by the metabolism of living organisms (Vasu, *et al*., 2009). Most of the bioactive compounds from various plants have been experimented to inhibit various microorganisms *in vitro* (Cowan, 1999). Thus knowledge of plant chemical compounds is important with the reason being that the information can aid in production of complex chemical substances (Mojab *et al*., 2003; Parekh, *et al.*, 2007; Parekh, *et al.*, 2008).

2.4.1. Uses of Medicinal Plants

The WHO recorded medicine for pharmaceutical industries from 119 plants and almost 75% are used in recent medicine as ways that corresponded exactly to how the indigenous people use the plant as medicine (Shulz *et al*., 2001). The usage of some herbal medicine differs far from each other based on the people's location and believes as well as their cultural values. The traditional

uses are mostly attributed to superstition, since traditional people do not have the scientific knowledge to explain how the plants are able to cure diseases. An instance to this concept is the Doctrine of signatures on the perception that the plant's appearance may give a gist of its medicinal activities (Van Wyk & Wink, 2004).

In African continent, there has been people to plant interaction from ancient days, which Africa is identified by rich group plants which forms almost 15% of human species and contains one of the most continental plants that is believed to be between the range 50,000 to 70,000 plant taxa (Klopper *et al*., 2002; Smith & van Wyk, 2002; Nigro *et al.,* 2004). African floral is not only characterised by its great variety but also its distinctiveness which is as large as 88% of floral species are that are often found at a particular place. A large number of endemic plant species has shown that most of the worldwide plant resources are uniquely from Africa and are purposely for medicine, horticulture, agriculture, forestry and other activities (Davis *et al*., 1994; Nigro *et al*., 2004). African indigenous medicine is noted to be among worldwide used medicine and the most different of all medicinal systems, which almost 70% of the Africans refer to herbalists with their health problems. The herbalist mostly diagnoses and treats the psychological basis of sickness after which they prescribe medicines for the management of the illness (van Wyk & Wink, 2004). An individual plant might possess bitter substances which stimulate digestion, anti-inflammatory compounds which reduce swelling and pain, phenolic compounds that works as antioxidants and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that aid in getting rid of waste product and poisons and alkaloids that improves mood and provides sense of well-being (van Wyk & Wink, 2004). Apart from plant chemical constituents being directly used for curing diseases, they can also serve as templates for drugs manufacturing (Eloff, 1998). Synthesizing potent components of medicinal plants might be poisonous in a very small

dose compare with a whole plant extract that becomes only poisonous when consumed in large quantities (Eloff, 1998).

2.4.2. Medicinal Plants with Antimycobacterial Activity

In recent years, there has been a rapid increase in the antiviral, antifungal, antibacterial and antituberculosis properties from different medicinal plants isolation of chemical components from all over the world. Evaluation of plant phytochemicals for anti-mycobacterial properties is normally tested using *Mycobacterium* culture in various agar and broth based media (Newton *et al*., 2000). There have been a lot of studies on the *in vitro* prevention of *Mycobacterium* species with medicinal plants. Important reports have been published on anti-mycobacterial natural product for the past decade. In spite of the fact that, there has not been any discovery of new drugs from isolations from plants in the market for the curing of tuberculosis, but some important chemical constituent have been discovered for different microbial disease (Cantrell *et al*., 1999). According to Mitscher & Baker (1998), tryptanthrin, erygibisoflavone, phaseollidin, erythrabyssin II, berberine and Lichoisoflavone which are plant-derived compounds could serve as potential anti-mycobacterial isolations and compounds from 123 medicinal plants.

Okunade *et al*., (2004), discussed 88 synthetic analogues and naturally occurring from marine organisms, plants and fungi that exhibited significant properties in the *in vitro* bioassays contrary to *Mycobacterium tuberculosis* and other mycobacterial species. Pauli *et al*., (2005) reviewed the current developments in myco-bacteriology and newly introduced of natural product chemistry tools and their ability to affect the initial procedures of finding new tuberculosis drug. About 70% of Indian medicinal plant species which comprise 255 out of 365 from different families had

exhibited anti-mycobacterial properties as described by Gautam *et al*. (2007). Surprisingly, when *in vitro* preliminary screening test was conducted, 149 species demonstrated positive ethno-medicinal in relation to the indigenous knowledge in treating tuberculosis and its related diseases (Gautam *et al*., 2007).

According to Grange and Davey (1990), the growth of *Mycobacterium tuberculosis* sensitive strain H37Rv was inhibited by ten out of the 408 ethanol extracts of plants such as *Guauacum officinale, Ipomea purge, Angustura vera, Cinnamonium camphora, Actaea spicata, Piper cubeba*, and *Rhamnus cathartica* at dilution of 1 in 1280. At lower dilutions a great number of the remaining extract also prevented the growth of the strain (Grange & Davey, 1990).

2.5. Hypothesis and motivation of study

Natural product investigation will be providing leading structures of different types which could serve as basis in the development of current and future drugs by the pharmaceutical industry. Most of the investigated plants in this studies, have exhibited very promising activity of mycobacterial effects on strains from *Mycobacterium tuberculosis* complex. Most of the plants species have also been found to be strong against different types of micro-organisms. Among the higher plants species of more than 250 000 species worldwide, only a few percentage of about

5% to10% have their phyto-constituents being investigated (Ayensu & De Filipps, 1978; Nahrsted, 2002) and an even smaller fraction has been submitted to biological or pharmacological screenings (Hostettmann, *et al*., 2002). The plant kingdom up till now has abundance of drugs remaining to be identified. Thus, the discovery of new antibacterial, antiHIV and anti-tuberculosis compounds from herbal remedies could assist in the development of new preparation to combat infectious diseases. Various research have revealed that the existence of the secondary metabolite compounds

form medicinal and physiological activities of the plants in the treatment of different illness (Sofowora, 1993). For example, a lot of herbal medicines that are rich in tannins have been tested to show its antibacterial properties against different microorganisms (Doss *et al*., 2009). Although saponins are haemolytic on red blood cell, they have no side effect when drink into the body and have important characteristics of reducing cholesterol level in the human system (Amos-Tautua *et al*., 2011). It has been tested to show that alkaloids contain both anti-bacterial (Erdemoglu *et al*., 2007) and anti-diabetic activites (Costantino *et al*., 2003). Flavonoids are hydroxylated phenolic substances known to be produced by plants as response to microbial infection and have been tested to possess antimicrobial properties against wide range of miccrooganisms. The presence of secondary metabolites such as tannins, saponins, alkaloids and phenols, have been discussed to be exhibiting antibacterial activities in humans (Rojas, *et al*., 2006; Nikitina, *et al*., 2007; Udobi, *et al*., 2008).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Microorganism

Mycobacterium tuberculosis H37Rv and Mycobacterium bovis strains were obtained from the Chest clinic of the Korle-Bu Teaching Hospital in Accra through the Ghana National TB Control. *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* isolates were cultured on Lowenstein-Jensen medium and were left to grow aerobically within a period of 4-8 weeks at 37^{o}C.

3.1.2. Plant Materials

Xylopia aethiopica	*Zingiber officinale*
Plate 1 (E1)	Plate 2 (E2)

Allium cepa

Plate 3 (E3)

Allium sativum

Plate 4 (E4)

Tetrapleura tetraptera

Plate 5 (E5)

Lantana hispida

Plate 6 (E6)

Bidens pilosa Plate 7 (E7)	*Phyllanthus fraternus* Plate 8 (E8)

Alchornea cordifolia Plate 9 (E9)

Different parts of nine (9) selected medicinal plants species, representing eight (8) different families, were sampled during the major dry season from different locations in Ghana.

Approximately, 10.0 kg of the fresh plants materials of each species was air-dried and ground. The plants used included: *Xylopia aethiopica* (Dunal) A. Rich *(Hwentia/Hwenteaa), Alchornea cordifolia* (Schum & Thonn.) Muell. Arg *(Egyama/Ogyama), Zingiber officinale (*Roscoe) *(Ginger/Akakaduro), Lantana hispida (*Kunth) (*Ananse dokon*), *Tetrapleura tetraptera* Taub. *(prekese), Allium cepa* var. aggregatum *(Anyaw), Allium sativum* L. *(Sara anwiw/Garlic), Phyllanthus fraternus* Webster *(Wabo wo mma agu wakyi/nkukubro/nkatseha) and Bidens pilosa* var. minor*(kurofidie).*

3.1.3. Chemical Reagent

Magnesium sulphate (Mahavir chemical Industries, India), magnesium citrate (Shreeji Pharma. International, India), asparagines (Qingdao Fraken International Trading, India), 70% ethanol, Potassium hydrogen- phosphate (America Elements), Malachite green (Matrix Pharma. Chemical, India), glycerol, isoniazid, Ziehl's Neelsen, and 10% Dimethyl Sulphate Oxide (DMSO) all obtained from A.B Enterprises, India., Phosphate Buffered Saline (PBS), Mayer's reagent, Millon's reagent, Ninhydrin, Fehling A and Fehling B reagents, Benedict's reagent, Molisch's reagent, concentrated H_2SO_4 , iodine solution, 2% solution of $FeCl_3$, Magnesium ribbon, concentrated HCl, 2% solution of NaOH, and acetic acid were all obtained from the Nouguchi Memorial Institute for medical Research and Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR).

3.1.4. Medium:

Lowenstein Jensen egg media, locally prepared at KCCR, were used for the various plant extracts.

3.2. METHODS

3.2.1. Plant Extraction

The dried powdered plant materials were extracted with 70% ethanol. One hundred and fifty grams (150g) of each plant material of each was extracted with 750 ml of 70% ethanol over three days. The extracts were filtered and concentrated to dryness at low pressure with rotary evaporator at 40^{o}C. The dry extracts were taken to the Centre for Plant Medicine Research for freeze drying and phytochemical screening.

3.2.2. Qualitative Phytochemical Constituent Analysis

All the extracts were screened to identify the bioactive chemical components using standard methods (Harborne, 1973; Trease and Evans, 1989; Sofowra, 1993)

3.2.2.1. Test for Phenols and Tannins

The plants extract (0.5mg) was dissolved in 2 ml of 2% Iron III chloride solution. A blue-green colouration represented the presence of tannins and phenols.

3.2.2.2. Flavonoids test

Shinoda test

The plants extract (0.5mg) was mixed with few pieces of Mg ribbon. Then concentrated hydrochloric acid was gently added to it. The appearance of a bright pink colour within few minutes showed the occurrence of flavonoids in the mixture.

3.2.2.3. Saponins test

The plants extract (0.5mg) was mixed with 5ml of distilled water in a test tube and then swirled strongly. The formation of foam for about 5 minutes was regarded as an evidence for the presence of saponins.

3.2.2.4. Glycosides test

Liebermann's test

The plants extract (0.5mg) was dissolved in 2ml each of trichloromethane and acetic acid. The solution was put on ice to cool. Gently, the concentrated tetraoxosulphate VI acid was poured into the mixture. A change in colour from violet to blue and later to green represented the occurrence of steroidal nucleus which is aglycone part of glycoside.

Salkowski's test

The plants extract (0.5mg) was dissolved in 2ml each of trichloromethane and later concentrated tetraoxosulphate VI acid with gentle swirling. A reddish brown colour represented the presence of steroidal ring which is aglycone part of the glycoside.

Keller-kilani test

The plants extract (0.5mg) was dissolved in 2ml of glacial acetic acid containing 2 drops of 2% solution of Iron III chloride. The solution was transferred into different test tube containing 2ml of concentrated tetraoxosulphate VI acid. At the interphase, there was brown ring which meant the occurrence of cardiac glycosides in the mixture.

3.2.2..5. Steroids test

The plants extract (0.5mg) was mixed with 2 ml of trichloromethane. Then concentrated tetraoxosulphate VI acid was poured onto the sideways. The appearance of red colour in the lower trichloromethane surface indicated the presence of steroids.

3.2.2.6. Terpenoids test

The plants extract (0.5mg) was mixed with 2ml of trichloromethane and later evaporated to dryness. To the mixture, a 2 ml of concentrated tetraoxosulphate VI acid was added and warmed for few minutes. The turning of the solution into gray showed that the solution contained terpenoids.

3.2.2.7. Alkaloids test

The plants extract (0.5mg) was dissolved in 2ml of 1% hydrochloric acid and then warmed under low heat. Mayer's reagent was added to the solution. The cloudy of the resulting precipitate was taken as result showing alkaloids presence.

3.2.3. Anti-mycobacterial Activity of Extracts on Mycobacterium tuberculosis

Each ethanol extract was dissolved in 2 ml of 10% Dimethyl sulphur oxide (DMSO) to obtain a stock solution of 50.0 mg/ml. The stock solution (2 ml) of each plant extract was dispensed in sterile Lowenstein-Jensen medium to obtain 5 mg/ml diluted solution. The isolates were dissolved in PBS and compared with Mcfarland standard and poured into sterilized glass tubes with few glass beads. Fifty milliliters (50ml) of Lowenstein-Jensen was added to the solution.

The isolated strain was inoculated on the Lowenstein-Jensen medium containing a single plant extracts. A Lowenstein -Jensen medium containing Isonazid (INH) served as the positive control whilst DMSO and Lowenstein-Jensen drug free medium were also cultured separately serving negative control. The test tubes having growth on Lowenstein-Jensen medium after four to eight weeks culturing at 37 ^{0}C were recorded. The minimal inhibitory concentration (MIC) of the alcohol extracts was established by adding varying concentrations (1.0-5.0 mg/ml) of every extract into test tube that already contains the cultured media. Before it became solidified, 5.0 ml of Lowenstein-Jensen medium were dispense with 5 ml, 2 ml and 1 ml plant extracts separately aseptically to all the test tubes and swirled gently until all the media solidified. The isolated bacteria were swirled in a circular pattern on the each Lowenstein-Jensen medium containing the plant extracts, before incubating at 37^{o}C for four to eight weeks. All the extracts were tested at 5.0 mg/ml, 2.5 mg/ml, and 1.0 mg/ml. Two blank test tubes containing Lowenstein-Jensen medium with isoniazid (INH) served as positive control set up, two drug free Lowenstein-Jensen medium and two Lowenstein-Jensen medium containing 10% DMSO with no plants extract served as negative control set up. The MIC was recorded as the lowest concentration of the plants extract

that did not allow any clear growth of *Mycobacterium tuberculosis*. The test was done in replications.

3.2.4. Dilution susceptibility testing against Mycobacterium tuberculosis

The plant extracts were evaluated against *Mycobacterium tuberculosis sensitive H37Rv and Mycobacterium bovis* applying the dilution procedure. The alcohol extracts were mixed in 10% DMSO in Lowenstein-Jensen medium to obtain a stock solution of 50.0 mg/ml. Two serial fold dilutions of each sample were evaluated were made with Lowenstein-Jensen medium to yield 5mg/ml with final decreasing concentration ranging from 5.0 to 1.0 mg/ml.
The cultured test tubes were then sealed and incubated at 37ºC for eight weeks. The MIC of the extracts were determined through the addition of 0.2µg/ ml Isoniazid (INH), 10% DMSO, individual plants extract and formulations from plant extracts separately and incubated at 37ºC for eight weeks.

Procedure for staining

Fixed TB slides were arranged on a staining rack and then flooded with Ziehl-Neelsen carbol fuchin. The flooded slides were heated for five minutes until they streamed. Each slide was then rinsed with water until all the free stains were washed away. Each slide was then flooded with decolourising solution for three minutes and rinsed thoroughly with water. Thereafter, slides were flooded with counter stain and left for five minutes. The slides were again rinsed thoroughly with water. Excess water was drained from the slides. Smears were allowed to air dry and then observed under a microscope.

CHAPTER FOUR

4.0 RESULTS

4.1. PHYTOCONSTITUENTS OF THE SCREENED MEDICINAL PLANTS

Table 1 shows the various phytochemicals present in the selected plants under study. Terpenoids were found in *Xylopia aethiopica, Zingiber officinale* and *Phyllanthus fraternus* while Phenols were found in *Xylopia aethiopica, Alchornea cordifola, Lantana hispida* and *Tetrapleura tetraptera.* Tannins were found in *Xylopia aethiopica, Zingiber officinale, Alchornea cordifola, Tetrapleura tetraptera, Phyllanthus fraternus, Bidens pilosa* and *Allium cepa* whilst flavonoids were present in all the selected plants except *Phyllanthus fraternus*. Steroids were found in the *Xylopia aethiopica, Tetrapleura tetraptera, Allium sativum, Phyllanthus fraternus,* and *Bidens pilosa.* Saponins were found in *Xylopia aethiopica, Zingiber officinale, Lantana hispida, Tetrapleura tetraptera, Allium cepa* and *Bidens pilosa* whilst glycosides were present in *Xylopia aethiopica, Zingiber officinale, Allium cepa* and *Bidens pilosa*. Alkaloids were found in *Xylopia aethiopica, Zingiber officinale, Alchornea cordifola, Tetrapleura tetraptera, Allium sativum* and *Phyllanthus fraternus*

Table 1: Phyto-constituents of the selected medicinal plants extracts

Plants	Terpenoids	Phenols	Tannins	Flavonoids	Steroids	Saponins	Glycosides	Alkaloids
Xylopia aethiopica	+	+	+	+	+	+	+	+
Alchornea cordifola	-	+	+	+	-	-	-	+
Zingiber officinale	+	-	+	+	-	+	+	+
Lantana hispida	-	+	-	+	-	+	-	–
Tetrapleura tetraptera	-	+	+	+	+	+	-	+
Allium cepa	-	-	+	+	-	+	+	–
Allium sativum	-	-	-	+	+	-	-	+
Phyllanthus fraternus	+	-	+	-	+	+	-	+
Bidens pilosa	-	-	+	+	+	+	+	–

Key: +=Present; - =Absent

4.2. ANTIMYCOBACTERIAL ACTIVITY OF TEST EXTRACTS

In this study, 10^{-2}mg/ml of *Mycobacterium bovis* (BCG) was inoculated on Lowenstein-Jensen drug free medium which was used as the negative control for eight weeks. From Figure 1, it could be seen that, there was no sign of growth for the first two weeks but 35 and 36 isolates were counted for weeks 3 and 4 respectively. The growth increased from 36 colonies to 64 colonies on the fifth week while the sixth and seventh weeks recorded 108 and 135 respectively. The final week (week 8) for the culturing also saw massive growth (168 colonies) of the *Mycobacterium bovis.*

In another set-up,10^{-2}mg/ml of BCG was innoculated on Lowenstein-Jensen egg media in which 2ml of 10% of DMSO was added and cultured for eight weeks and also used as a negative control. During the eight weeks of culturing, there were no sign of growth on both duplicate tubes for two weeks, after which growth started on the third week with 21 colonies and 32 colonies on the fourth week. The fifth week recorded 42 colonies whilst the sixth also recorded 50 isolated colonies of *Mycobacterium bovis*. Both seventh and eight weeks saw very massive increase in growth of 105 and 163 isolated colonies respectively.

Isoniazid (INH) of concentration 0.2µg/ml was used as a positive control set-up. The INH showed no sign of growth for the first four weeks, however five (5) colonies were observed for the next two weeks which then increased to 10 colonies for another two weeks.

The test Extract coded E_1, was obtained from the pod of *Xylopia aethiopica*. The experiment was done in triplicates and the average growth was calculated for each extract. For the first two weeks, there was no sign of growth. On the third week, 21 colonies were observed whilst the fourth, fifth and sixth weeks recorded 31, 38 and 45 colonies respectively. The seventh week recorded 55 colonies with the eight week showing 65 colonies.

The Extract E_2 was obtained from *Zingiber officinale*. Mycobacterial growth was not observed for the first two (2) weeks of the study but, the third week showed growth of 31 colonies while the fourth week recorded 44 colonies. Weeks five, six, seven and eight had 49, 65, 110 and 138 colonies respeectively. In the case of Extract E_3 from Allium *cepa*, no growth was observed for the first two weeks of study. The third week showed growth of 22 colonies and, 35 colonies in fourth week. The fifth weeek also recorded 46 colonies, the sixth week had 78 colonies, the seventh week also showed 110 colonies whilst the eight week recorded 157 colonies.

Extracts E_4 extracted from *Allium sativum* inhibited the growth of the *Mycobacterium bovis* throughout the eightweeks.

Extract E_5 was the extract from *Tetrapleura tetraptera* which recorded no growth for the first two weeks of the study. The third and fourth weeks respectively showed growth of 20 and 23 colonies with the fifth and the sixth showing 42 and 49 colonies whilst the seventh week recorded a growth of 77 colonies. The eight week recorded a growth of 130 colonies.

Extract E_6 was the extract from the plant *Lantana hispida* and recorded zero growth for first seven weeks of culturing, however, four (4) colonies were, observed on the eighth week. Extract I_7 was extracted from *Bidens pilosa*. The first two weeks showed zero growth but on the third week 15 colonies were observed whilst 23 colonies were observed on the fourth week. There was growth of 37 colonies on the fifth, 47 colonies on the sixth week, 59 colonies on the seventh with eighth weeks recording 119 colonies.

Extract E_8 extracted from *Phyllanthus fraternus* record zero growth for the first two weeks with the third week showing growth 24 colonies. The fourth week recorded 34 colonies, 41 colonies for the fifth week, 65 colonies in sixth week, 104 colonies in the seventh week whilst the eighth week recorded 159 colonies.

Extract E_9 was extracted from *Alchornea cordifolia* and recorded zero growth in the first two weeks but growth started showing from the third week to the eighth week. The third week showed growth of 24 colonies, fourth week recorded 40 colonies, fifth week recorded 48 colonies, sixth week also recorded 61 colonies whilst the seventh and eight week recorded 110 colonies and 157 colonies respectively.

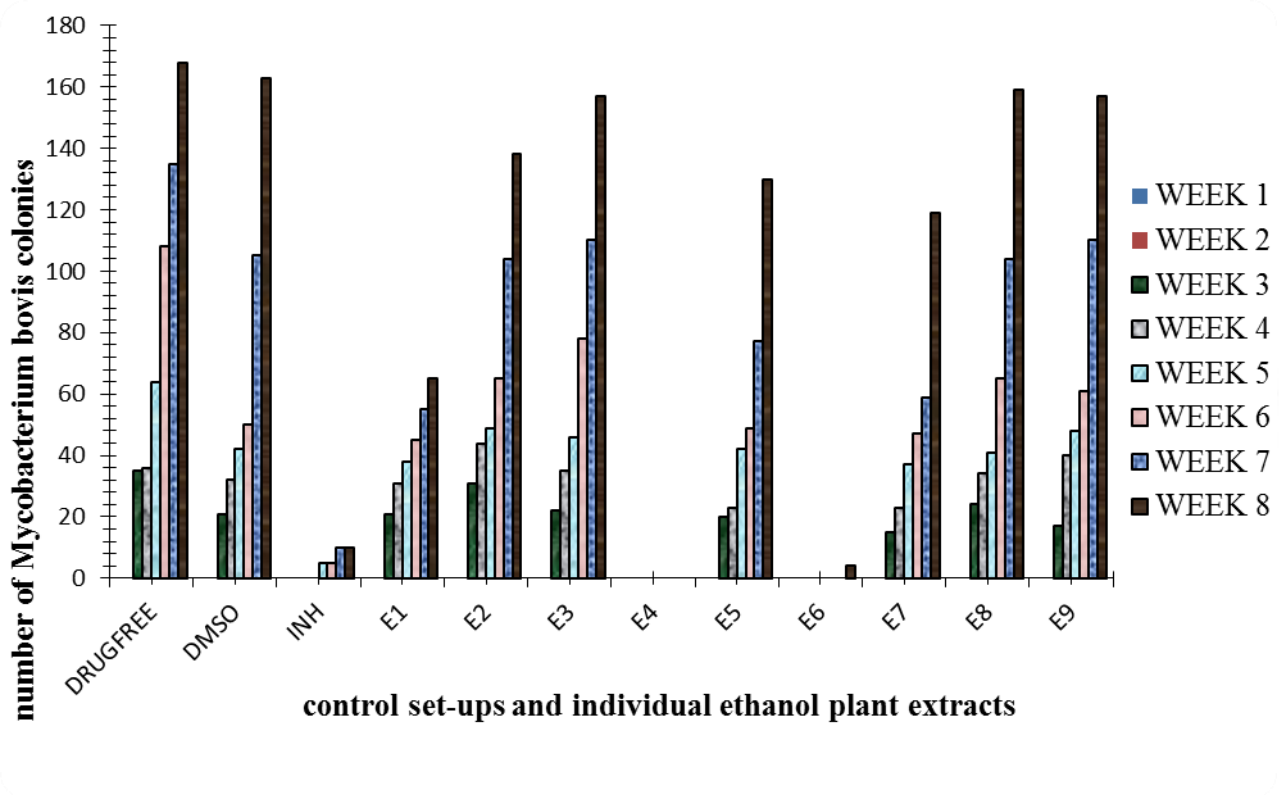

Figure 1: Susceptibility of *Mycobacterium bovis* to the controls and the ethanol extracts

From the results obtained from the individual plants, formulations were prepared from some of the extracts with very good antimycobacterium activities. Other combinations prepared according to the usage by some Traditional Herbalists were also analyzed.

Formulation-1 (F-1) was obtained from *Tetrapleura tetraptera*, *Allium sativum*, *Bidens pilosa* and *Xylopia aethiopica*. In the first three weeks no growth was observed but the fourth week recorded 20

colonies. Fifth week observed growth with 23 colonies whilst the sixth, seventh and eighth weeks recorded a growth of 30, 35, and 38 colonies respectively.

Formulation-2 (F-2) was obtained from *Lantana hispida*, *Xylopia aethiopica*, *Zingiber officinale*, and *Allium sativum*. There were zero growth for the first five weeks but, the sixth week recorded 16 colonies whilst the seventh and eighth weeks recorded 25 and 29 colonies respectively.

Formultion-3 (F-3) was combination of *Allium sativum* and *Lantana hispida* and recorded no mycobacterial growth during the eight week period (Figure 2).

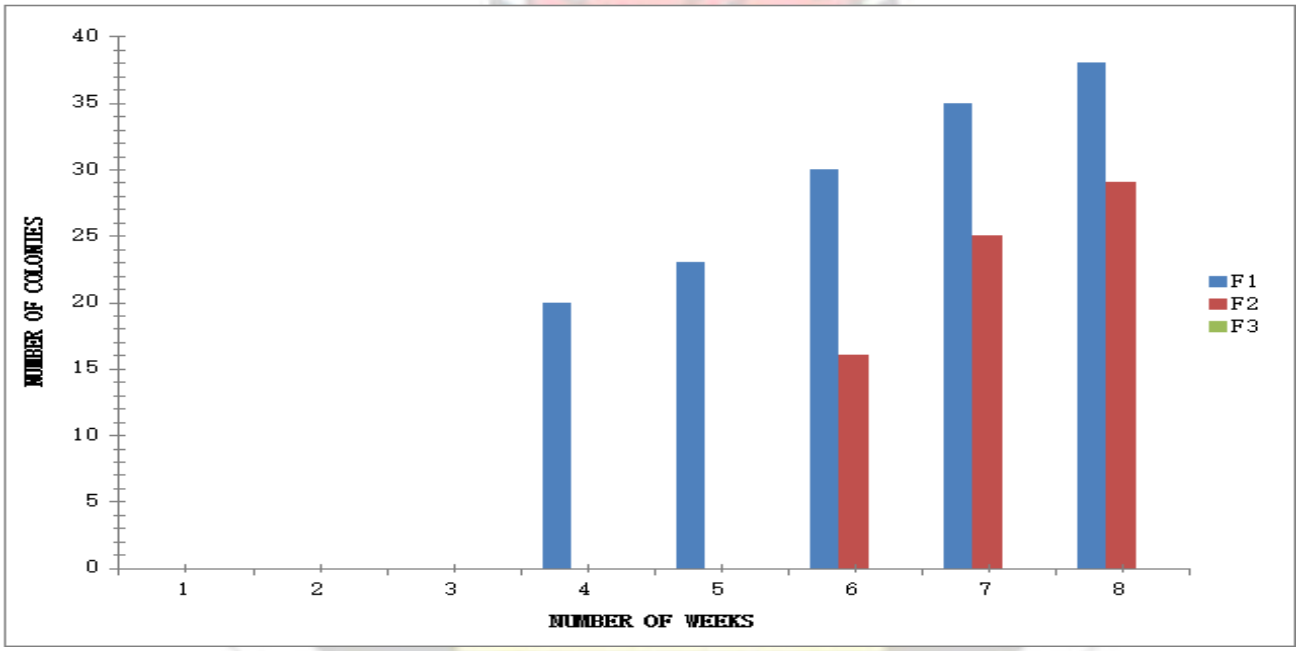

Figure 2: Susceptibility of *Mycobacterium bovis* to the three Formulations

The concentration of individual plants was reduced to 2.5 mg/ml using the same positive and negative control set-up.

Extract E_1, recorded no growth for the first two weeks; but the third week recorded 25 colonies.

There were 38 colonies on the fourth week, 48 colonies in the fifth week whilst the sixth, seventh and eighth weeks recorded 76 colonies, 125 colonies for both seventh and eighth weeks

respectively.

Extract E_2 observed no growth for the first two weeks but the third week recorded 27 colonies, fourth and fifth recorded 34 and 48 colonies respectively, with the sixth week recording 79 colonies, the seventh and eighth weeks all recording 127 and 169 colonies respectively.

Extract E_4 inhibited the growth of *Mycobacterium bovis* during the entire eight week periods of culturing. Extract E_6 observed no growth for the first seven weeks but the eighth week recorded 7 colonies. Extract E_7 recorded no growth for the first two weeks; the third week recorded 10 colonies. The fourth and fifth week observed 20 and 33 colonies respectively, with the sixth and seventh weeks recording 41 and 57 colonies whilst 124 colonies were observed on the eight week (Figure 3).

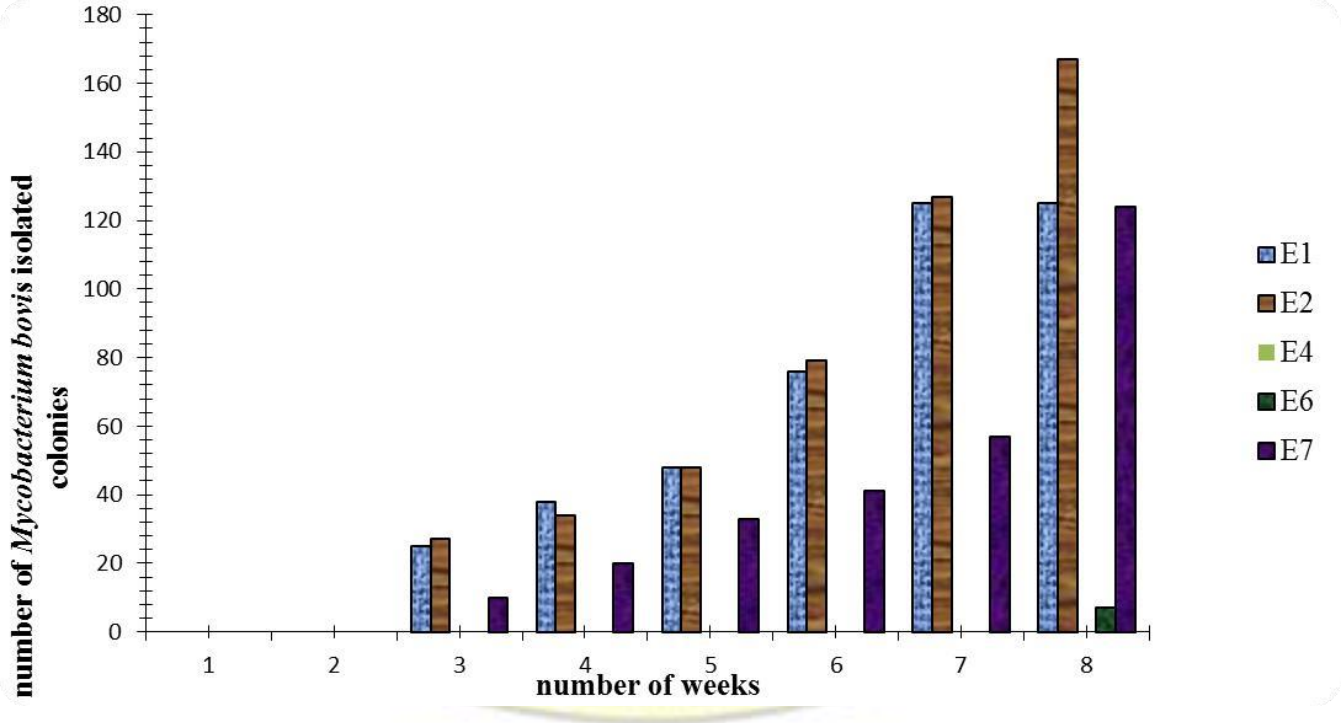

Figure 3: Susceptibility of *Mycobacterium bovis* to the plant extracts at 2.5mg/ml

Formulation-1 (F-1) is the formulation from the plants *Bidens pilosa*, *Tetrapleura tetraptera*, *Allium Sativum* and *Xylopia aethiopica*. *Xylopia aethiopica* was added because in Ghana, herbalists and citizens usually use it in the treatment of cough and other respiratory diseasees. *Tetrapleura tetraplera* was also used since the local herbalists in Ghana use it during drug preparation for treating microbial infections. Formulation-1 (F-1) recorded no growth for the first three weeks. The fourth week recorded 20 colonies whilst there were 23 and 25 colonies on the fifth and sixth weeks respectively. The seventh and eighth weeks recorded 33 and 38 colonies respectively.

For Formulation-2 (F-2), no growth was observed for the first four weeks, 4 colonies on the fifth week, whilst the sixth, seventh and eight weeks recorded scanty colonies of 20 colonies, 25 colonies, and 30 colonies respectively. Formulation-3 (F-3) is a formation from *Allium sativum* and *Lantana hispida* and inhibited the growth of the *M. bovis* throughout the eight week period of study (Figure 4).

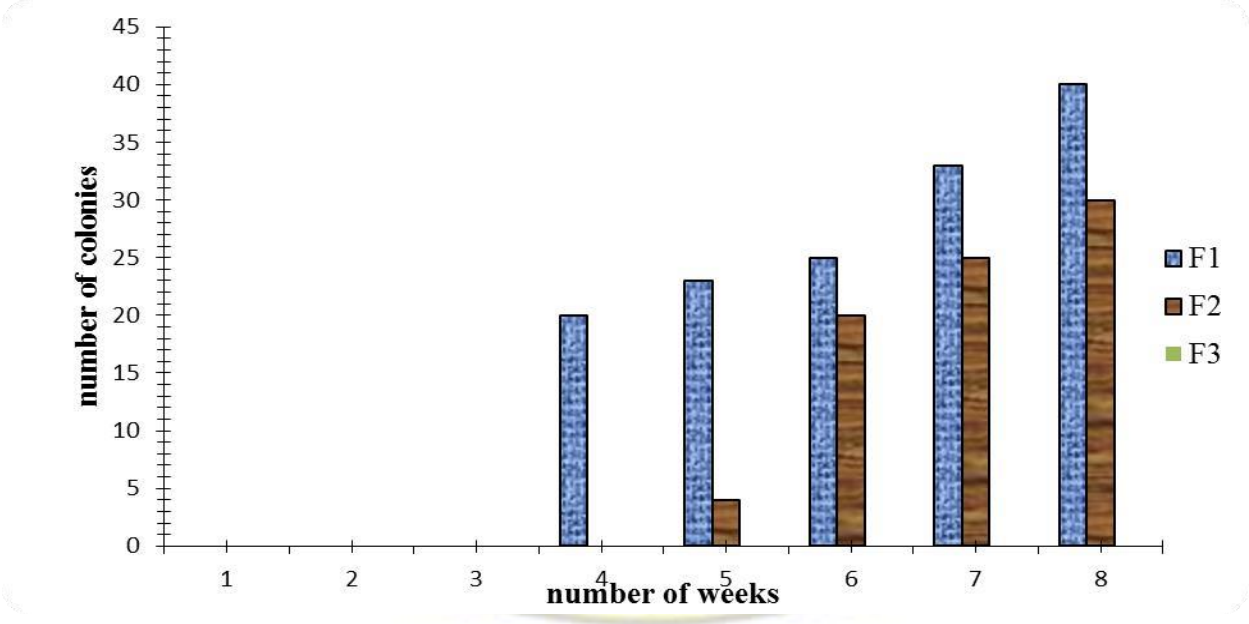

Figure 4: Susceptibility of *Mycobacterium bovis* to the three Formulations

The concentration of individual plants was further reduced to 1.0 mg/ml using the same positive and negative control-set-up. Extract E_1 recorded no growth for the first two weeks but; there were 27 colonies on third week, 38 colonies on fourth week, 49 colonies on the fifth, 82 colonies on the sixth week whilst the seventh and the eight weeks observed 125 and 130 colonies respectively. Extract E_2 recorded no growth for the first two weeks whilst the third and fourth weeks recorded 28 and 34 colonies respectively. There were 51 colonies on fifth week, 89 colonies on sixth week, 127 on the seventh week and 172 colonies on the eighth week. Extract E_4 inhibited the growth of *M. bovis* throughout eight period of the study. Extract E_6 recorded no growth for the first seven weeks with eight week recording 10 colonies.

Extract E_7 recorded no growth for the first two weeks with the third week recording 10 colonies whilst the fourth week and the fifth week recorded 22 colonies and 31colonies respectively. The sixth week recorded 44 colonies with the seventh week and the eighth week recording 79 colonies and 103 colonies respectively (Figure 5).

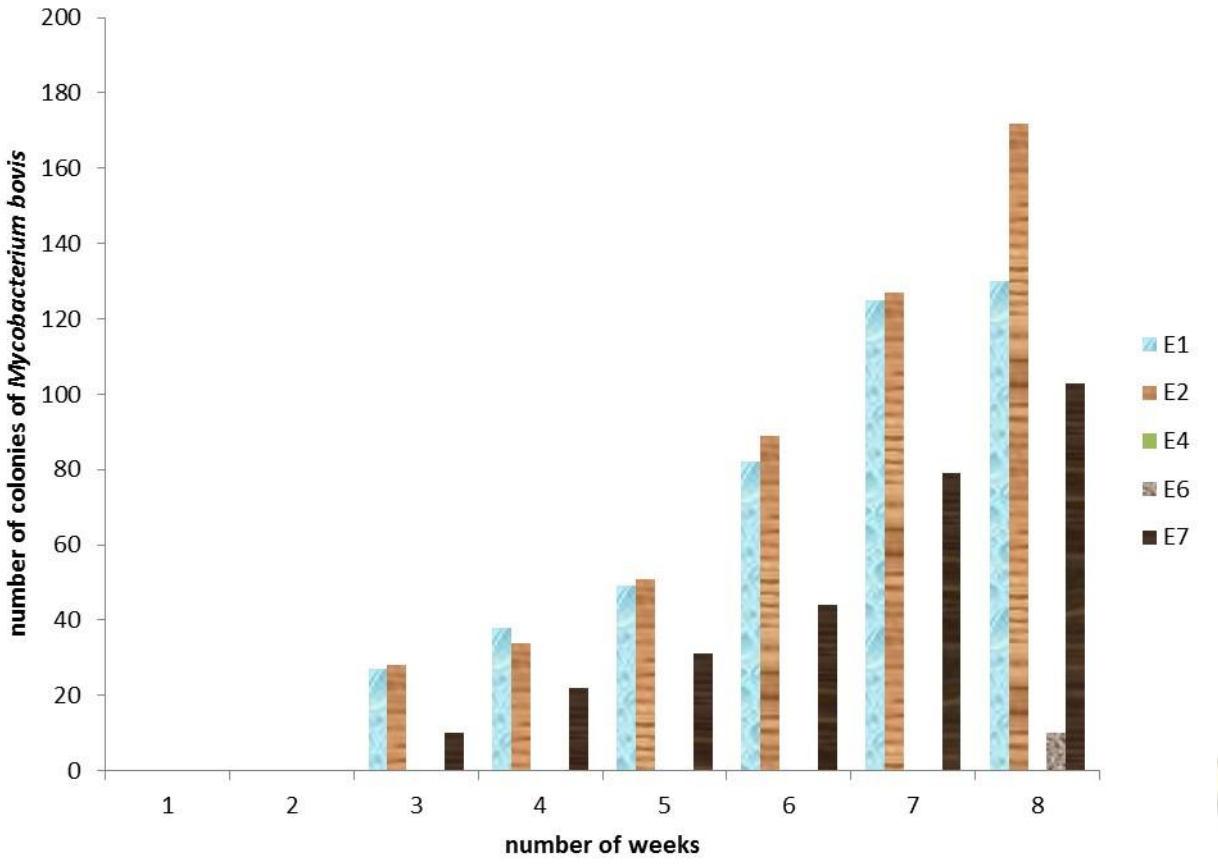

Figure 5: Susceptibility of *M. bovis* to the plant extracts at 1.0mg/ml

Formulation-1 (F-1) recorded no growth for the first three weeks but there were 22 colonies, 23 colonies and 27 colonies on fourth, fifth and sixth week, with the seventh week recording 35 colonies. The eight week recorded 41 colonies. Formulation-2 (F-2) observed no growth for the first four weeks, 9 colonies on fifth week, 21 colonies and 28 colonies on sixth and seventh week respectively with the eighth week recording 35 colonies. Formulation-3 (F-3) inhibited the growth of *M. bovis* throughout the eight weeks of culturing (Figure 6).

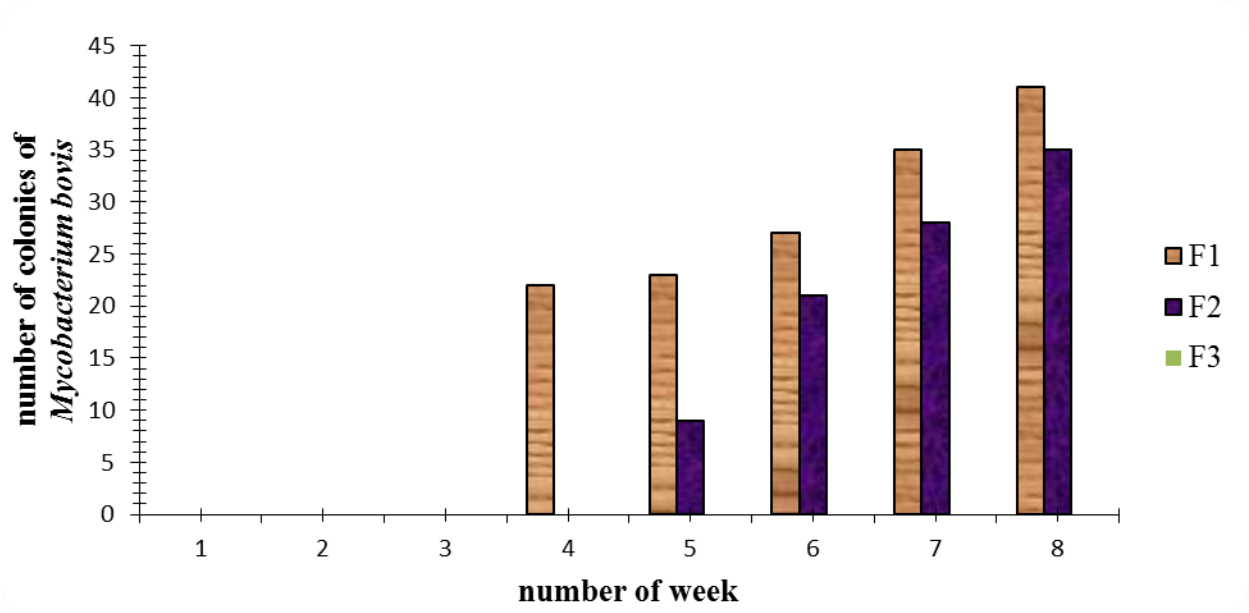

Figure 6: Susceptibility of *Mycobacterium bovis* to the three Formulations

4.3. ANTIMYCOBIAL ACTIVITY OF TEST EXTRACTS AGNAINST MYCOBACTERIUM TUBERCULOSIS H37RV STRAIN.

In this study, the Lowenstein-Jensen (LJ) drug free medium with 10^{-2} mg/ml *M. tuberculosis* H37Rv strain recorded no growth for the first two weeks, with third week and the fourth weeks recording 40 colonies and 59 colonies respectively. The fifth week recorded more than 100% increment (127 colonies) in growth. The sixth, seventh and the eighth weeks recorded respectively 154 colonies, 168 colonies and 179 colonies. The 10% DMSO as a negative control recorded no growth for the first two weeks. The third, fourth, fifth, sixth, seventh and eighth week recorded; 40 colonies, 59 colonies, 127 colonies, 154 colonies, 168 colonies and 179 colonies respectively. 0.2µg isoniazid (INH) inhibited the growth of *Mycobacterium tuberculosis* H37Rv strain throughout eight weeks of culturing (Figure 7).

For the individual plant extract, Extract E_1 extracted from *Xylopia aethiopica* had no growth for the first three weeks with the fourth week recording 4 colonies whilst, the fifth week observed 21 colonies, with 29 colonies, 45 colonies and 60 colonies for the sixth, seventh and eight weeks respectively. Extract E_2 extracted from *Ziniger Officinale* had no growth for the first two weeks with the remaining six weeks of culturing recording 11 colonies, 23 colonies, 37 colonies, 63 colonies, 138 colonies and 159 colonies on the third, fourth, fifth, sixth, seventh and eight weeks respectively.

Extract E_3 extracted from *Allium cepa* had no observable growth recorded for the first three weeks of the study, however, there were 28 colonies, 35 colonies, 61 colonies, 110 colonies and 118 colonies for the fourth, fifth, sixth, seventh and eight weeks respectively. Extract E_4 was the extract from *Allium sativum*, and inhibited the growth of *Mycobacterium tuberculosis* H37Rv strain for the eight weeks under investigation. Extract E_5 was extracted from *Tetrapleural tetraptera* and had no growth for the first three weeks with; the fourth week recording 22 colonies. The fifth week recorded 27 colonies, with the sixth week observing 35 colonies whilst seventh week and eight weeks recording 44 colonies and 65 colonies respectively.

Extract E_6 was the extract from *Lantana hispida*. The first six weeks of culturing recorded no growth, with seventh week recording 20 colonies whilst the eight week observed 22 colonies. Extract E_7 was the extract from *Bidens pilosa* which observed no growth for the first three weeks.

The fourth week recorded 25 colonies whilst there were 31 colonies, 36 colonies, 40 colonies and 45 colonies for the fifth, sixth, seventh and eighth week respectively. Extract E_8 from *Phyllanthus fraternus* recorded no growth for the first two weeks with 19 colonies recorded on third week. The fourth week recorded 31 colonies, the fifth week recorded 64 colonies, the sixth week recorded 82 colonies whilst the seventh week and eighth weeks recorded 112 colonies respectively. Extract E_9 was extracted from *Alchornea cordifolia* with the first two weeks recording no growth. The third

week recorded E6 colonies with 28 colonies on fourth week, 36 colonies on fifth week, and 89 colonies for the sixth week. The seventh week recorded 116 colonies whilst the eight week recorded 164 colonies.

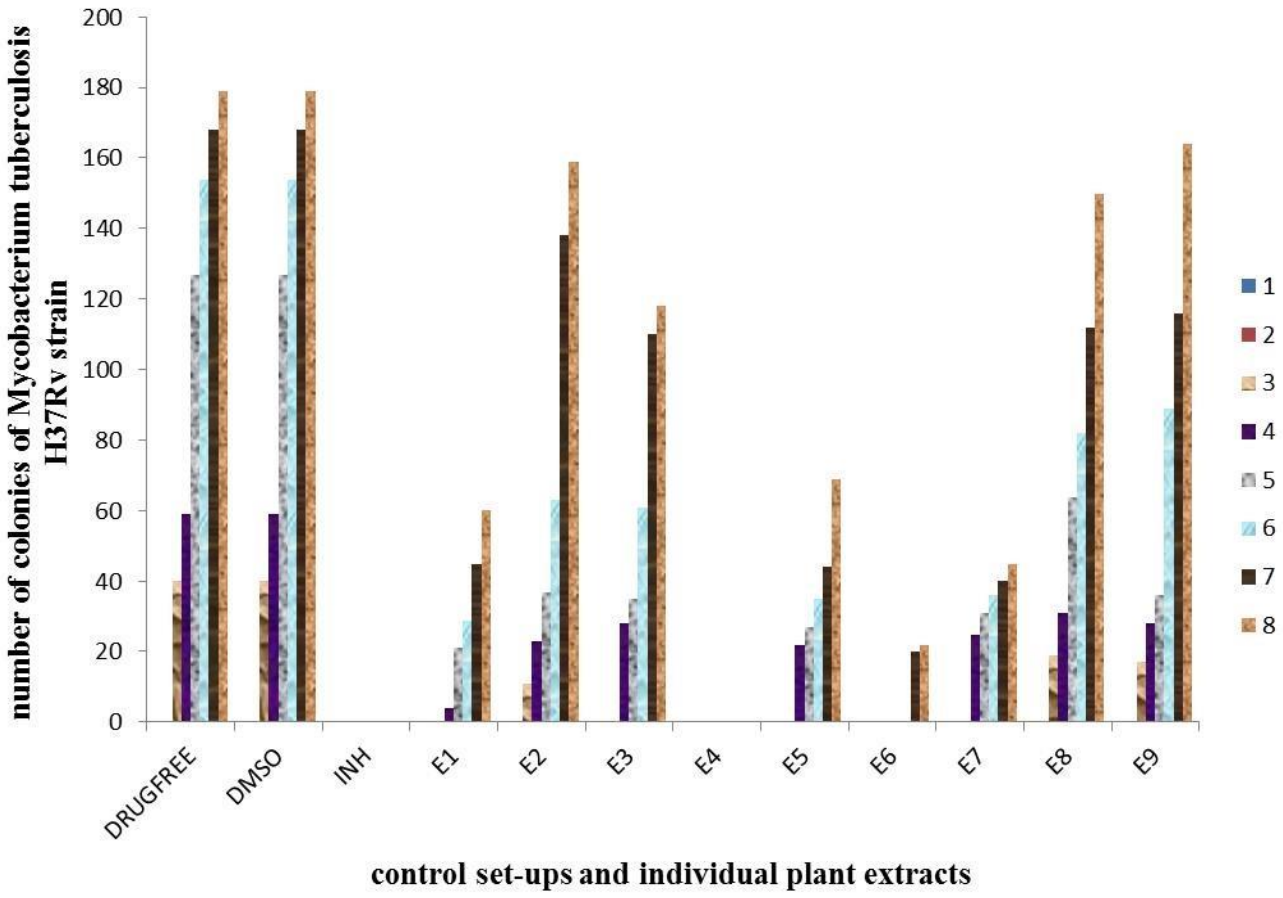

Figure 7: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts at 5.0mg/ml

Formulation-1 (F-1) is the extraction from *Bidens pilosa, Tetrapleura tetraptera, Xylopia aethiopica and Allium sativum*. Formulation-1 (F-1) recorded no growth for the first four weeks with the fifth week observing of 21 colonies. There were 23 colonies on sixth week, 29 colonies on the seventh week, and 35 colonies on the eighth week. Formulation-2 (F-2) was a Combination of *Lantana hispida, Allium sativum, Zingiber officinale and Xylopia aethiopica.* Formulation-2 (F-

2) recorded no growth for the first seven weeks, with the eighth week recording 22 colonies. Formulation-3 (F-3) was a preparation from *Allium sativum* and *Lantana hispida* and was able to prevent the growth of *Mycobacterium tuberculosis* H37Rv strain during the eight weeks of culturing.

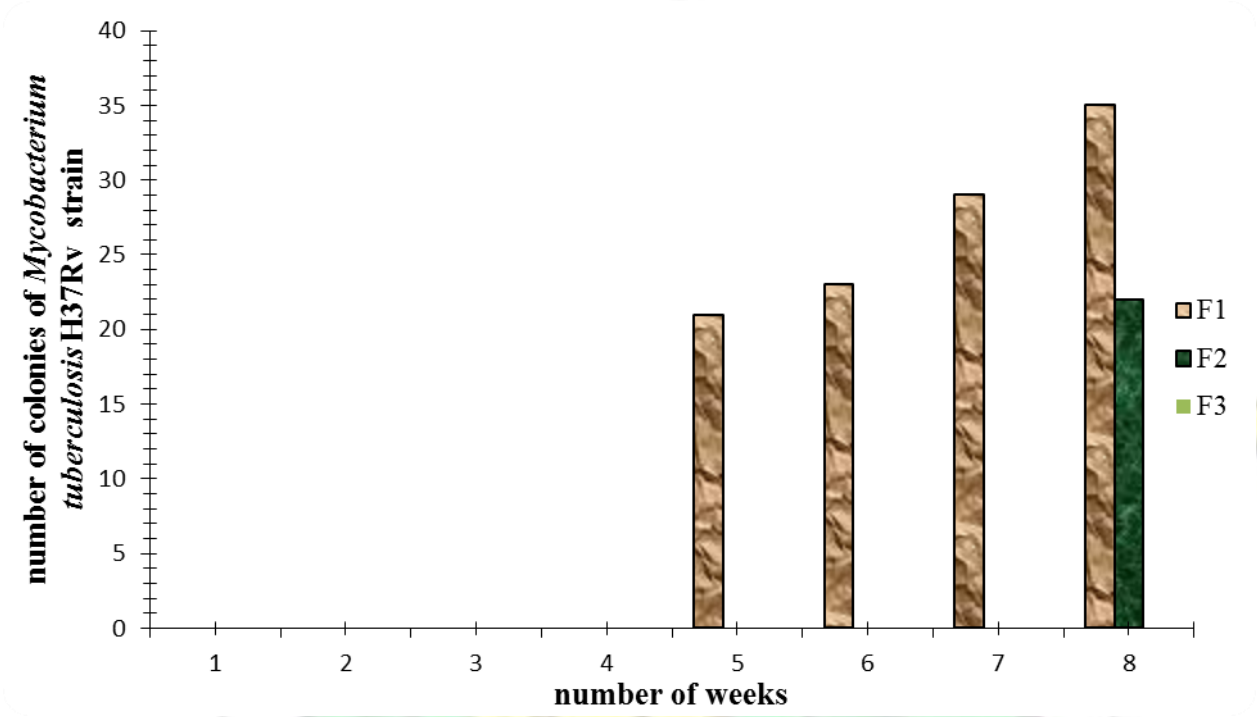

Figure 8: Susceptibility of *M. tuberculosis* H37Rv strain to the three Formulations.

The extracts with promising activity were further diluted from the 5.0mg/ml to 2.5mg/ml. Extract E_1 was a preparation from *Xylopia aethiopia*, and recorded no growth for the first three weeks with the fourth week recording 28 colonies. There were 33 colonies and 44 colonies on fifth week and sixth week respectively whilst the seventh week observed 67 colonies; with the eight week observing 89 colonies.

Extract E_2 was extracted from *Zingiber officinale* and produced no growth for the first two weeks. The third week recorded 16 colonies; the fourth week recorded 27 colonies, with the fifth week, sixth week, seventh week, and eight week recording 47 colonies, 80 colonies, 145 colonies and 185 colonies respectively. Extract E_4 extracted from the *Allium sativum* inhibited the growth of *M. tuberculosis* H37Rv strain throughout the eight weeks of culturing.

Extract E_6 extracted from *Lantana hispida*, recorded no growth for the first five weeks of culturing; with the sixth, seventh and eight weeks also recording 20 colonies, 23 colonies, and 25 colonies respectively. Extract E_7 was prepared from *Bidens pilosa*. The first three weeks recorded no growth whilst there were 29 colonies on fourth week, 35 colonies on fifth week, 37 colonies on sixth week, 45 colonies on the seventh week, and 63 colonies recorded for the eighth week.

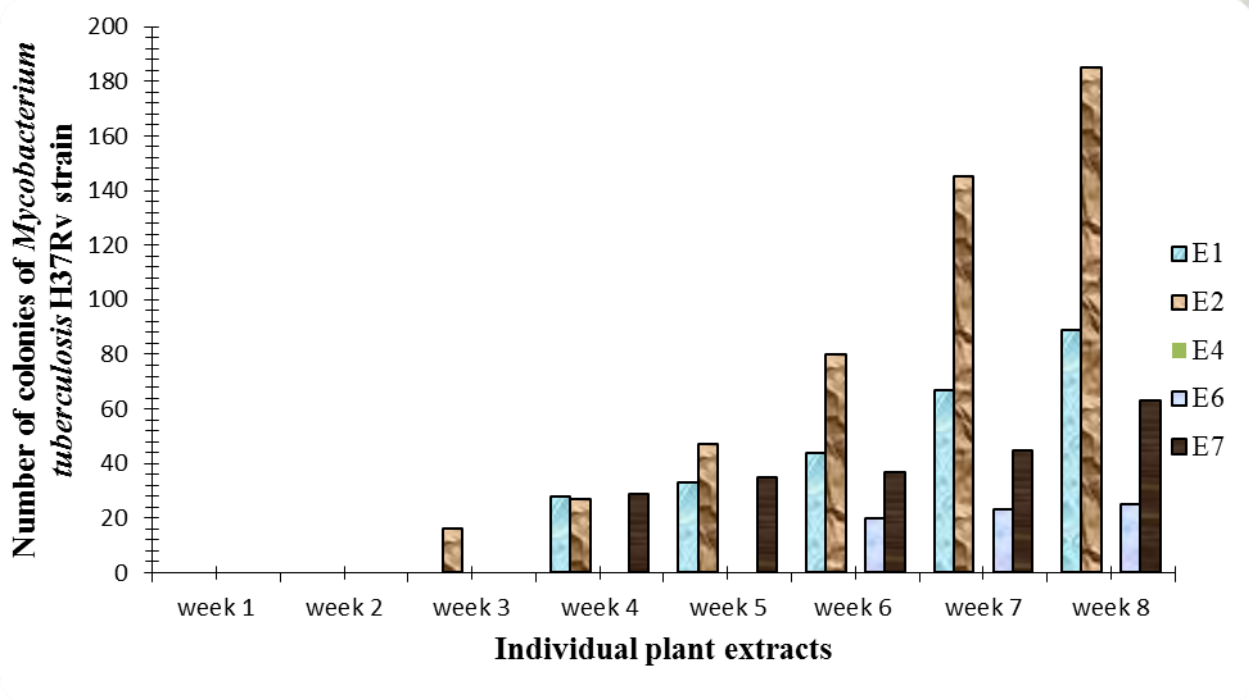

Figure 9: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts 2.5mg/ml

Formulation-1 (F-1) extracted from *Bidens pilosa, Tetrapleura tetraptera, Xylopia aethiopica* and

Allium sativum, recorded no growth for the first four weeks, however the fifth week recorded 24 colonies, with the sixth week and seventh week recording 31 colonies and 37 colonies respectively, whilst the eighth week recorded 38 colonies. Formulation-2 (F-2) extracted from *Lantana hispida, Allium sativum, Zingiber officiale* and *Xylopia aethiopica* recorded no growth for the first seventh week with the eighth week recording 25 colonies.

Formulation-3 (F-3) extracted from *Lantana hispida* and *Allium sativum* inhibited the growth of *M. tuberculosis* H37Rv strain throughout the eight weeks of culturing.

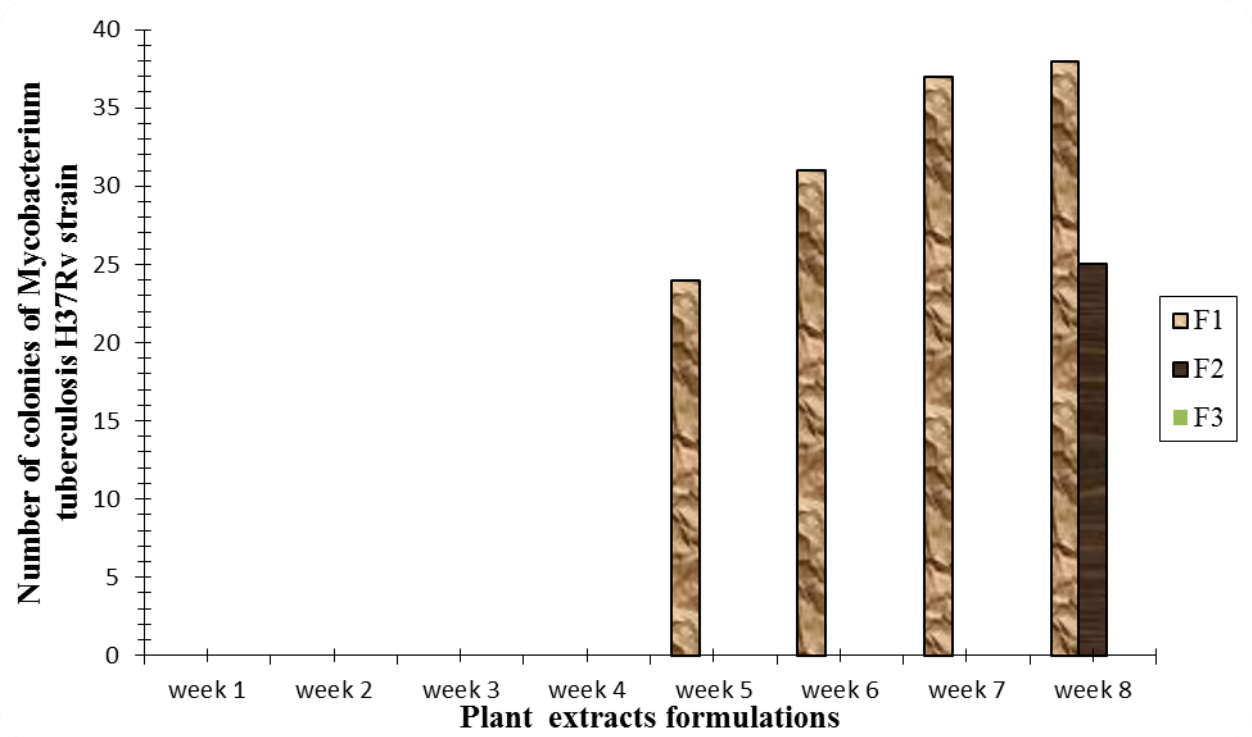

Figure 10: Susceptibility of *M. tuberculosis* H37Rv strain to the three Formulations

The concentration of each potent extract was further reduced to 1.0mg/ml and the antimycobacterial activity again analysed. Extract E_1 prepared of *Xylopia aethiopica* recorded no growth for the first three weeks whilst the fourth week recorded 28 colonies with the fifth, sixth, seventh and eight weeks recording

34 colonies, 42 colonies, 57 colonies and 97 colonies respectively. Extract E_2 was extracted from *Zingiber officinale*. There was no growth for the first two weeks but; the third week recorded 18 colonies, 27 colonies on fourth week, 47 colonies on fifth week, with the sixth week and seventh week recording 85 colonies and 148 colonies whilst the eight week recorded 193 colonies. Extract E_4 extracted from *Allium sativum* inhibited growth on the Lowenstein-Jensen egg medium *Mycobacterium tuberculosis* during the eight weeks under investigation.

Extract E_6 extracted from *Lantana hispida* observed no growth for the first five weeks of culturing. The sixth week recorded 21 colonies; the seventh week recorded 25 colonies, with the eighth week recording 27 colonies. Extract E_7 prepared from *Bidens pilosa* observed no growth for the first three week, and 29 colonies on fourth week. The fifth week recorded 38 colonies, with the sixth week recording 42 colonies, whilst the seventh week and eighth week observed 47 colonies and 56 colonies respectively.

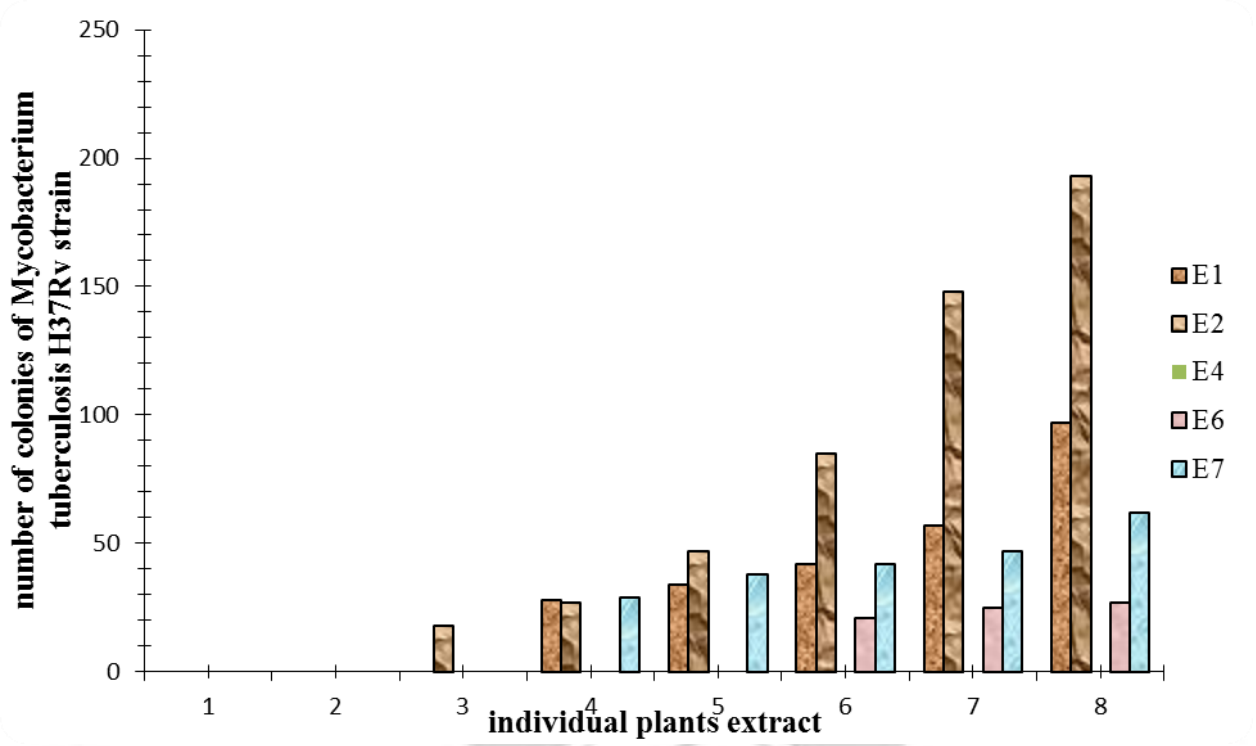

Figure 11: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts at 1.0mg/ml

Formulation-1 (F-1) extracted from *Bidens pilosa, Tetrapleural tetraptera, Allium sativum* and *Xylopia aethiopica* recorded no growth for the first four weeks. The fifth week observed 25 colonies with the sixth, seventh and eight weeks recording 31 colonies, 37 colonies, 38 colonies respectively. Formulation-2 (F-2) was the extract from *Lantana hispida, Allium sativum, Zingiber officinale and Xylopia aethiopica*. There were no growth for the first seventh week withthe eighth week recording 28 colonies. Formulation-3 (F-3) made from a combination of *Alluim saltivum and Lantana hispida* inhibited growth on the Lowenstein-Jensen egg medium containing 10^{-2} mg/ml of H37Rv for the eight week duration of the study.

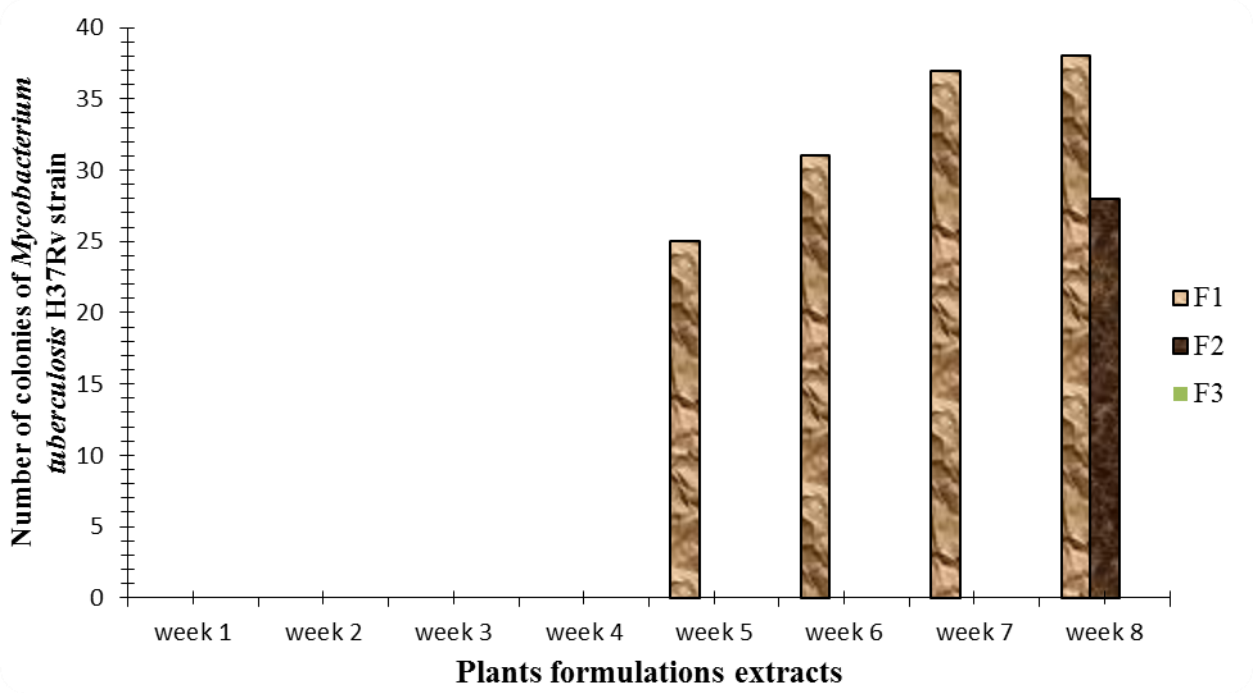

Figure 12: Susceptibility of *M. tuberculosis* H37Rv strain to the three Formulations

CHAPTER FIVE

5.0. DISCUSSION

The indigenous use of medicinal plants in Ghana has well been documented; however, the effectiveness of most of these plants has not been scientifically evaluated. For instance, infectious disease cases such as TB and HIV/AIDS are quite prevalent in Ghana, particularly in the rural areas, where an astounding number and variety of plants are used by communities to treat these diseases without prior scientifically determined information. In this investigation, nine medicinal plants were evaluated against *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv.

Phytochemical analysis conducted on the various herbal medicine extract showed the existence of phytoconstituents, including tannins, saponins, alkaloids, phenols, flavonoids, steroids, glycosides and terpenoids. Various research have revealed that the existence of these compounds form

medicinal and physiological activities of the plants in the treatment of different illnesses (Sofowora, 1993). For example, a lot of herbal medicines that are rich in tannins have been tested to show its antibacterial properties against different microorganisms (Doss *et al*., 2009). Although saponins are haemolytic on red blood cell, they have no side effect when drink into the body and have important characteristics of reducing cholesterol level in the human system (Amos-Tautua *et al*., 2011).It has been shown that alkaloids contain both anti-bacterial (Erdemoglu *et al*., 2007) and anti-diabetic activities (Costantino *et al*., 2003). Flavonoids are hydroxylated phenolic substances known to be produced by plants as response to microbial infection and have been tested to possess antimicrobial properties against wide range of miccrooganisms. The presence of secondary metabolites such as tannins, saponins, alkaloids and phenols, have been evaluated to be performing antibacterial activities in humans (Rojas, *et al*., 2006; Nikitina, *et al*., 2007; Udobi, *et al*., 2008). The antibacterial activities exhibited by the extracts from the nine selected plants, in this study, may perhaps be due to the presence of tannins, saponins, alkaloids, flavoid, terpenoid, steroid, glycosides and phenols identified in this study.

The positive control drug used was Isoniazid (INH), which is a front line drug for the treatment of tuberculosis (TB). A result is considered to be "no growth" when medium produces growth of colonies less than 20 whilst growth colonies between 20 and 40 is considered to be "scanty growth". Specifically, 20 to 29 colonies is considered as "scanty 1", 30-39 colonies is considered as "scanty 2" while 40 to 49 colonies is considered "scanty 3". Growth showing 50 to 100 colonies is considered as "plus one (+) growth", 101 to 150 colonies is considered as "plus two (+ +) growth", growth colonies above 151 upward colonies is also considered as "plus three (+++) growth" and when there is a confluent growth, as in negative controls, it is considered as "plus four (++++) growth".

In this study, *Xylopia aethiopica* showed positive growth of plus one (+) at concentration of 5mg/ml against *Mycobacterium bovis*, and the number of colony growth increased as the plant concentration was reduced to 2.5mg/ml and 1.0mg/ml to plus two(++) on both concentration. When the same plant extract was used against *Mycobacterium tuberculosis H37Rv* at the same various concentrations, similar results were obtained but the number of colonies increased slightly at the various different concentrations. *Xylopia aethiopica* was used in the various formulations against the *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv at concentrations of 5mg/ml, 2.5mg/ml, and 1.0mg/ml and this is because, in Ghana, herbalists (Personal communication) and the citizens usually use it in the treatment of cough and other respiratory diseases.

Onyeagba et al. (2004) found the synergistic effect of ethanol extract of ginger and garlic against Bacillus spp. and Staphylococcus aureus. They also found the antimicrobial activity of the ethanol extract of ginger, lime and garlic against broad range of bacteria including Bacillus spp., Staphylococcus aureus, Escherichia coli, and Salmonella spp. According Sebiomo, *et al* (2010), Zingiber officinale has the potentional of inhibiting the growth of *Staphylococcus aureus* and *Streptococcus pyogenes* but in this study, massive colony growth of plus three (+++) of *Mycobacterium bovis* and *Mycobacterium tuberculosis H37Rv* at the various concentrations of *Zingiber officinale* implies that *Zingiber officinale* has no effect on the two strains used for the investigation.

According Uchechi, *et al* (2010), Tetrapleura tetraptera extracts both cold water and hot water, have antimicrobial properties against *Escherichia coli, Staphyloccoccus aureus, Pseudomonas aeruginosa and Salmonella typhi* but in this study, *Tetrapleura tetraplera* did not showed positive

action against the two strains of *Mycobacterium tuberculosis* used but it was used in the formulations prepared because the local Herbalists in Ghana use it during drug preparation for treating microbial infections. *Allium sativum* inhibited the growth of both *Mycobacterium bovis* and *Mycobacterium tuberculosis H37Rv* at various concentrations used. In comparison, it was observed that *Allium sativum* did better than the isonazid (Figure 1). *Lantana hispida* also inhibited the growth of both *Mycobacterium bovis* and *Mycobacterium tuberculosis H37Rv* at various concentrations, comparable to the effectiveness of isonazid. From the investigation, it could be concluded that *Allium Sativum* and *Lantana hispida* have the property of inhibiting the growth of the *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv.

Based on the activities shown by some of the individual plants against the test microbes, they were formulated into various formulations and tested against the *Mycobacterium tuberculosis* H37Rv and the *Mycobacterium bovis* using the same concentrations as the individual plants. *Bidens pilosa*, *Allium cepa*, *Phyllanthus fraternus and Alchornea cordifolia* did not show any significant inhibition of the test strains used at the various concentrations.

The traditional herbal medicine users use all parts plant to suppress, manage, and cure diseases (Wijesekera, 1991). Almost 25% of the prescribed drugs used in America is made from at least one active compound or component obtained from herbal medicine, whiles some components from herbal medicine and some are manufactured to resemble and behave like a herbalism chemical components (Balick, 1990). Few of other herbalism and their chemical components are used, and is a guide during manufacturing current drugs (Farnsworth, 1984).

The results from this study corroborate, the importance ethnopharmacological surveys play in the selection of plants for bioactivity screening. The results obtained in this study, represent a worthwhile expressive contribution to the characterization of the anti-mycobacterial activities of

plant extracts of traditional medicinal plants from the Ghanaian flora. From the time of ancient, people all over the world were using herbal medicine as the only way of managing and treating many illnesses. Herbal medicine has been and still will be the main provider of medicines, as it has been since human existence (van Wyk *et al*., 1997). Studies have shown that just 30% to 40% of total plants in the plant kingdom are involved in current's conventional drugs; few of them are been served as nutritional supplements and more (Kirby, 1996; Hostettmann &

Marston, 2002).

Comparing activities of the three formulations to the growth of the test microbes, it can be deduced that two of the formulations (F-2 and F-3) were slightly active against the *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv while the F-3 inhibited the growth of both *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv. Many research institutions all the continents are interested in the testing of plants to know their biological activities, especially, with therapeutic potential. Most potential of higher herbal medicine as the basis for new medicines is unexplored (Hostettman *et al*., 1996). For instance, among more than 250,000 species of higher plants, only about 5-10% has been investigated chemically for the presence of biologically active compounds (Ayensu & De Filipps, 1978; Balandrin *et al*., 1993). In this study, the various plant formulations made had the potency or ability to prevent the growth of *Mycobacterium bovis* and *Mycobacterium tuberculosis* sensitive strain H37Rv at MIC of 1.0mg/ml. The demonstration of inhibition activities of the test plant extracts has revealed their value in a traditional medicine and supports the enormous role of medicinal plant in primary health care. Medicinal plants serve as important raw materials for primary health care, especially Africans including Ghana and other continents. The aims of using plants as the main therapeutic agents include: 1) separate bioactive chemical components for direct usage drug; 2) come out with bioactive compound of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher

properties and or lower poison; 3) serve as pharmacologic equipments; 4) usage of the every part of the plant or some aspect of it as a herbal remedy. According to Penna *et al*., (2001), ethnobotanical data are useful in the search for new antimicrobial agents with several bioactive compounds being isolated from medicinal plants

(Penna, *et al*., 2001).

Formulation-3 (F-3), comprising a combination of *Allium sativum* and *Lantana hispida* exhibited 100% inhibition against *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* even at a minimum concentration of 1.0mg/ml. This is, in fact, the first documented report on the combined activity of *Allium sativum* and *Lantana hispida* against tuberculosis-causing agents such as *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Rv. A combination of *Lantana hispida, Allium sativum, Zingber officinale* and *Xylopia aethiopica* also exhibited greater than 90% inhibition against the two test microbes. The individual plant extracts like *Allium sativum* and *Lantana hispida* also showed greater than 99% and greater than 85% inhibition against *Mycobacterium bovis* H37Rv and *Mycobacterium bovis* respectively while

Xylopia aethiopica, Zingiber officinale, Allium cepa, Tetrapheura tetraptera, Bidens pilosa, Phyllanthus fraternus and *Alchornea cordifolia* showed greater than 40% inhibitory activities against both *Mycobacterium bovis* H37Rv and *Mycobacterium bovis*. There are reports from other workers on the inhibition of Mycobacteria by medicinal plants. The compound allicin from *Allium sativum*, for instance, was found to be as potent as some of the standard anti-tubercular drugs such as streptomycin, isoniazid, ethanbutol and rifampicin (Jain, 1994). In another study,

Allicin extracted using ethanol, inhibited the growth of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium tuberculosis* TRC-C1193 that were completely resistant to Isoniazid with a minimum inhibitory concentration (MIC) of 70µg/ml for both organisms (Indian Council of Medicinal Research, 2004).

In this study, the crude extract of *Alluim sativum* was active against the standard *Mycobacterium tuberculosis* H37Rv and the *Mycobacterium bovis* at minimum inhibition concentration (MIC) of 1.0mg/ml while crude extract of *Lantana hispida* showed greater than 85% inhibitory property also at a concentration of 1.0mg/ml. It can be recorded that F-3 showed 100% inhibition property at a concentration of 5.0mg/ml down to 1.0mg/ml against the *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* with resistance defined as the lowest concentration of drug that inhibits growth with less than 20 colonies. Control experiment showed that the 10% DMSO in the media had no effect on the growth of the test microbes.

The ethanol extracts gave high percentage extract yield from all the selected medicinal plants which suggest that ethanol is a preferred solvent system for extracting the phyto-constituents in the selected medicinal plants. One out of the nine extracts from the selected medicinal plants, demonstrated appreciable inhibition as compared with the standard first-line drug employed in the study as another one showed slightly inhibition in variation as compared with isoniazid employed in the study. It can also be concluded that *Xylopia aethiopica and Zingiber officinale* have no positive activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis* as used by local people and herbalist in the treatment of tuberculosis and that they use that at the own risk and ignorant. These findings to a large extent agree with similar works carried out by Jain,

(1994), in India by India Council of Medicinal Research (2004), and in Nigeria by Anochie, *et al* (2011). This observation suggests that the selected medicinal plants are of a potential source of natural chemicals which might help in the inhibiting and killing of various members of the *Mycobacterium tuberculosis* complex. All extracts of the plants tested showed varying degree of anti-tubercular activities against the test *Mycobacterium tuberculosis* sensitive strain H37Rv and

Mycobacterium bovis. The ethanol extraction of *Allium sativum* showed higher properties against *Mycobacterium tuberculosis* sensitive strain H37Rv and *Mycobacterium bovis*, this could be due to the fact that, ethanol extracted more phyto-constituent which might be linked to its higher activity (Jain, 1994). The compound allicin from *Allium sativum* was found to be as potent as some of the standard antitubercular drugs such as Streptomycin, isoniazid, ethanbutol and rifampicin. In a study by the Indian Council of Medicinal Research (2004), allicin prepared from the ethanol inhibited the growth of Mycobacterium H37Rv and Mycobacterium TRC-C1193 that were completely resistant to isoniazid.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Ethanol extract of *Allium sativum* had the highest inhibition against *Mycobacterium tuberculosis H37Rv* and *Mycobacterium bovis* (MIC of 1 mg/ml), Ethanol extraction of *Lantana hispida* had a slight inhibition against the test micro-organism at both high and low concentrations while a formulation made up of *Allium sativum* and *Lantana hispida* had minimum inhibition concentration of 1mg/ml against *Mycobacterium tuberculosis* sensitive strain *H37Rv and Mycobacterium bovis*. *Xylopia aethiopica and Zingiber officinale* had no positive activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis* as used by local people and herbalist in the treatment of tuberculosis and other respiratory ailments. Local medicinal plants have high chance of managing tuberculosis faster than the existing drugs. The selected medicinal plants used for the study were evaluated and found out that they contain phenols, tannin, flavonoid, steroids, glycosides, terpenoids, saponins and alkaloids. The two most successful plants were found to contain phenols, flavonoids, saponins, steroids and alkaloids

6.2. Recommendations

The following are my recommendations:

- The active phyto-constituents present in the five medicinal plants with activity should be isolated to determine their anti-tubercular, antimicrobial and other antibacterial activities.
- The toxicity of the plant extracts should be studied in subsequent works
- Even though the plant extracts have been found to inhibit the growth of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* at certain concentrations, the minimum bactericidal concentrations (MBC) should be performed on the plants to ascertain whether they are bacteriostatic or bacteriocidal
- Further study should include other local medicinal plants to determine their potency and activity against multi-drug resistance tuberculosis to come out with a new drug for its treatment and management.

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