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ANTIMYCOBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS EXTRACTS IN THE MANAGEMENT OF MYCOBACTERIUM TUBERCULOSIS H37RV AND MYCOBACTERIUM BOVIS

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Abstract

Nine potent medicinal plants were selected through ethno-botanical survey in Southern parts of Ghana. These medicinal plants used to treat respiratory diseases, stomach ailment and other microbial infections were evaluated for anti-tubercular activity. These selected plants species were tested individual against drug sensitive strain of Mycobacterium tuberculosis (H37Rv) and Mycobacterium bovis at concentration ranging from 1.0 to 5.0 mg/ml using Lowenstein-Jensen egg medium at bacteriology department of Kumasi Centre for Collaborative Research in Tropical Medicine (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 organism at 5.0 mg/ml. This study has scientifically substantiate the used selected medicinal plants used in the treatment of tuberculosis in Ghana and also revealed scientifically that, there is high potential of these medicinal plants which can treat tuberculosis even better than standard drugs

Keywords: Antimycobacterial, Medicinal Plants, Mycobacterium bovis, Tuberculosis

Introduction

Recent years have witnessed a higher increase in the use and search for new drugs derived from plants. Tuberculosis has attracted the attention of Microbiologists, Ethnopharmacologists, Botanists, Natural-product chemists and others are all searching for new drugs which can cure fast and has no side effect. 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, thus, suffered a great deal from microbial diseases and continues to suffer from them.

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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.

Tuberculosis is an endemic and pandemic bacterial disease caused by the *Mycobacterium tuberculosis* complex. 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, S.K. & Mohan, A., 2004). Each year, an estimated eight million new cases and two million deaths occur due to TB worldwide (Kishore, P.V. et al., 2007). Treatment, prevention and the rate at which tuberculosis is spreading has been the concern of World Health Organization and individuals. Traditional herbal users make use the various parts plants to prevent, reduce, and manage illnesses (Wijesekera, R.O.B., 1991). These plants and their parts release various chemical substances that work on the body. In these moderns the awareness about 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 examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, P.M., 1996), antimicrobial drugs (Phillipson, J.D., & Wright, C.W., 1996) and anti-hepatotoxic compounds. The research was aimed at determining the susceptibility of *Mycobacterium tuberculosis H37Rv* and *Mycobacterium bovis* to extracts from the selected individual medicinal plants. There have been a lot of researches on the *in vitro* prevention of *Mycobacterium* species with medicinal plants. 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.

Materials And Methods

Mycobacterium tuberculosis H37Rv and Mycobacterium bovis strains were obtained from the Chest clinic of the Korle-Bu Teaching Hospital in Accra through the Ghana

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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 to ensure proper growth since the Mycobacterium strains growth and work effectively with human body temperature. 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. This was done in order to get the medicinal plants at the state of drying. Approximately, 10.0 kg of the fresh plants materials of each species was air-dried and grounded. 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) (*Anansedokon*), *Tetrapleura tetraptera* Taub. *(prekese), Allium cepa*var. aggregatum *(Anyaw), Allium sativum*L. *(Sara anwiw/Garlic),Phyllanthus fraternus* Webster *(Wabowommaaguwakyi/nkukubro/nkatseha) and Bidens pilosa*var. Minor *(kurofidie).*

The dried powdered plant materials were extracted with 70% ethanol over three days. The extracts were filtered and concentrated to dryness at low pressure with rotary evaporator at 40^{o}C to ensure that the ethanol evaporates leaving the various phytoconstituents intact. The dry extracts were taken to the Centre for Plant Medicine Research for freeze drying and phytochemical screening.

The individual ethanol plant extracts were each dissolved in 2ml of 10% Dimethyl sulphur oxide (DMSO) separately to obtain a stock solution with concentration of 50.0 mg/ml. A 2ml of the stock solution of plant extract was dispensed into sterile Lowenstein-Jensen medium to obtain 5mg/ml concentration of each extract then concentration was decrease to 2.5mg/ml and also to 1.0mg/ml. Each isolate was dissolved in PBS and compared with Mcfarland standard and poured into sterilised glass tubes with few glass beads.

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Results and Discussion

Phytoconstituents of The Screened Medicinal Plants

Various phytochemicals present in the selected plants under study are shown in the table below. Terpenoids were found in *Xylopia aethiopica*, *Zingiber officinale* and *Phyllanthus fraternus* while Phenols were found in *Xylopiaa ethiopica*, *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	-	+	-	+	-	+	-	_
Tetrapleurate traptera	-	+	+	+	+	+	-	+
Allium cepa	-	-	+	+	-	+	+	_
Allium sativum	-	-	-	+	+	-	-	+
Phyllanthus fraternus	+	-	+	-	+	+	-	+
Bidens pilosa	-	-	+	+	+	+	+	_

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Key: **+** represent presence of the phyto-chemical in the medicinal plant and **-** represents the absence of the phytochemical in the medicinal plant.

Antimycobial Activity of The Extracts Against *Mycobacterium Bovis*.

At the end of the eight weeks, it was observed that at concentration of 5mg/ml, *Lantana hispida* was able to inhibit the growth of *Mycobacterium bovis* for the first six weeks recording zero count growth and recorded only four isolated colonies by the eight week as compared to the first line TB drug; isoniazid which recorded five isolated colonies at six week and recorded ten colonies as at the eight week. Although, colonies recorded by both is considered as no growth but the extract showed more inhibition properties.

Allium sativum exhibited very great inhibitory property by recording zero growth count throughout the eight weeks of study. Comparing the growth *Mycobacterium bovis* on *Allium sativum* with the standard drug, it could be observed that *Allium sativum* has high inhibition property. *Xylopia aethiopica* also inhibited *Mycobacterium bovis* for the first three weeks, then saw a growth of 21colonies on the fourth week representing scanty one growth and 65 colonies representing plus one (+) growth on the eighth week. *Xylopia aethiopica* and *Tetrapleura tetraptera* all showed a sign of slight inhibition by inhibiting the growth of *Mycobacterium bovis* for the first three and four weeks respectively. Isolated colonies of 60 and 69 respectively were recorded on the seventh and eighth week, all representing plus one (+) growth. *Bidens pilosa* also observed an impressive inhibition property by inhibiting *Mycobacterium bovis* for the first three weeks and 45 isolated colonies representing scanty three growth on the eighth week.

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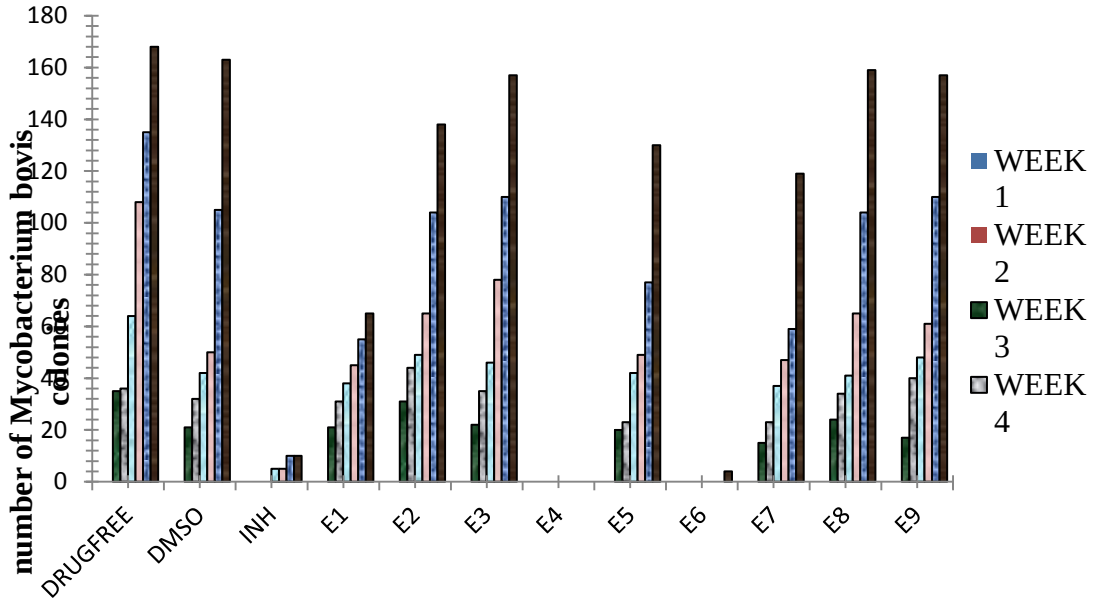

Figure 1: Susceptibility of *Mycobacterium bovis* to the controls and the ethanol extracts

At a concentration of 2.5mg/ml, *Xylopia aethiopica* also inhibited *Mycobacterium bovis* for the first two weeks, then showed a growth 25 colonies on the third week representing scanty one growth increasing growth up to 125 colonies on the eight week representing plus two (++). 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 been *Allium sativum* inhibited the growth of *Mycobacterium bovis* during the entire eight week periods of culturing. Extract E_6 is *Lantana hispida* and it also observed no growth for the first seven weeks but the eighth week recorded 7 colonies. Extract E_7 was the code for *Bidens pilosa* which recorded no growth for the first two weeks; the third week recorded 10 colonies. The fourth and fifth

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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.

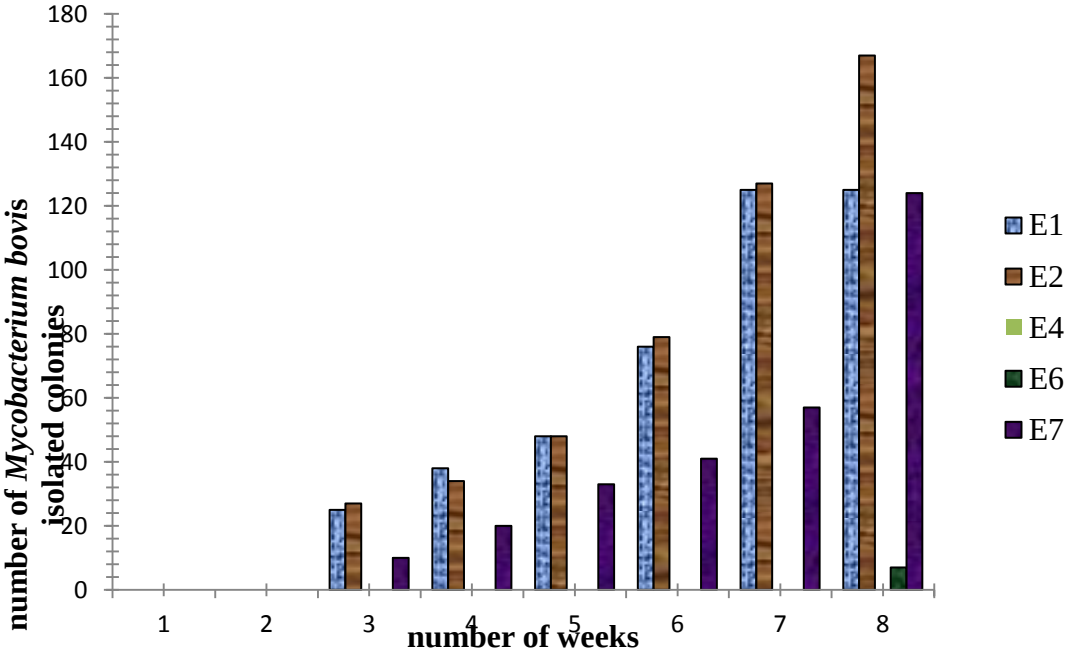

Figure 2: Susceptibility of *Mycobacterium bovis* to the plant extracts at 2.5mg/ml

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_2extracted from *Ziniger officinale* 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 *Mycobacterium 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

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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

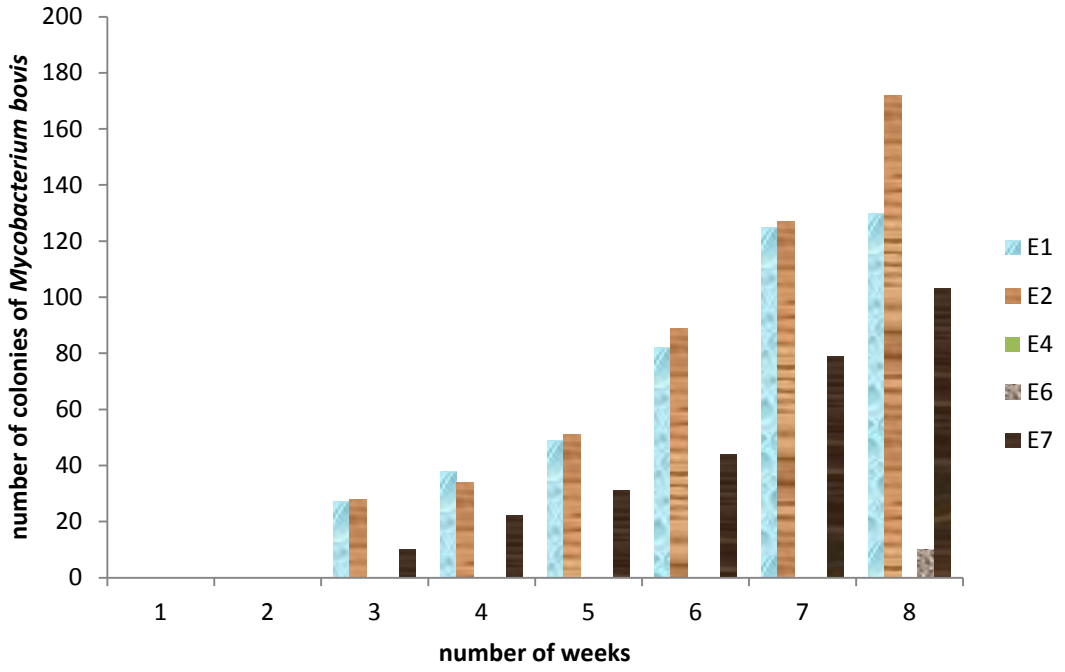

Figure 3: Susceptibility of Mycobacterium bovis to the plant extracts at 1.0mg/ml

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 *Mycobacterium 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. 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 *Tetrapleura 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.

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The third week recorded 17 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.

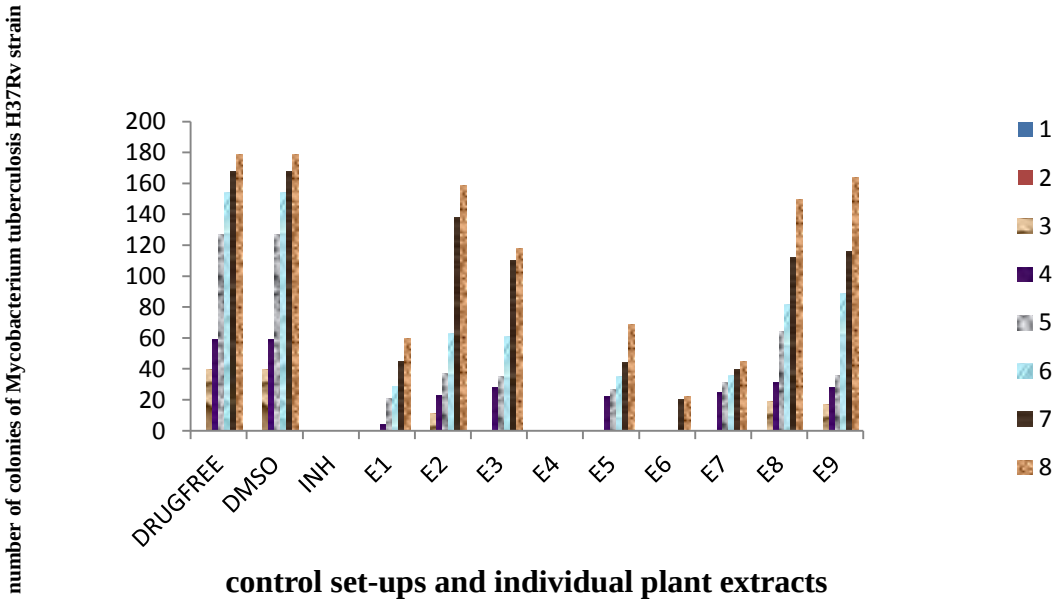

Figure 4: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts at 5.0mg/ml

At 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 and44 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

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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.

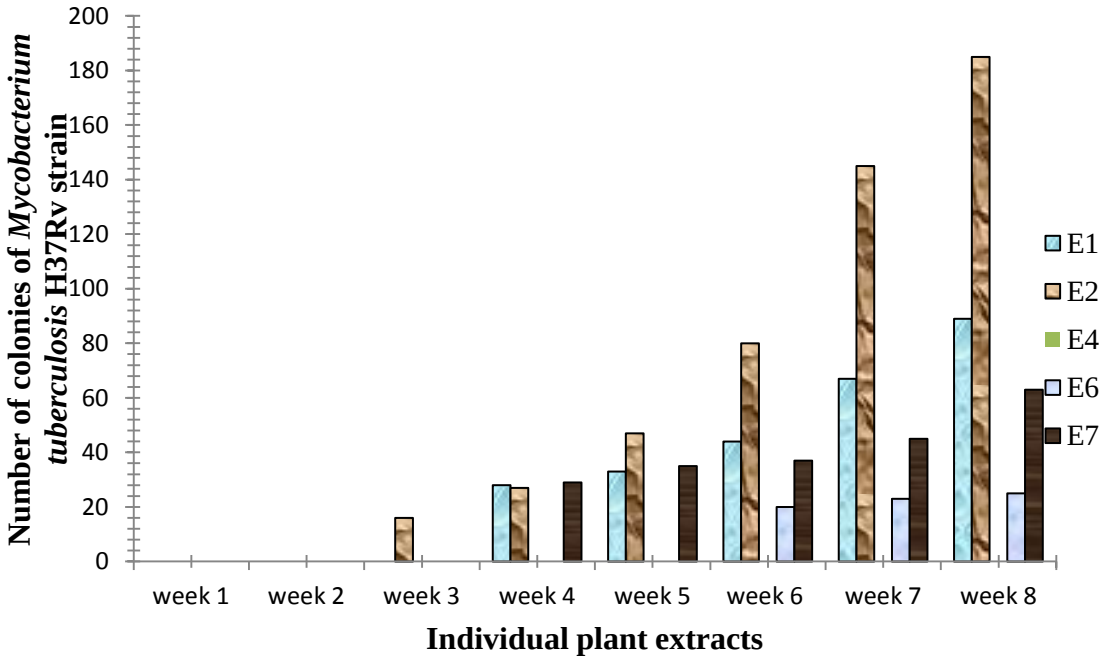

Figure 5: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts 2.5mg/ml

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The concentration of each potent extract was further reduced to 1.0mg/ml and the anti-mycobacterial 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.

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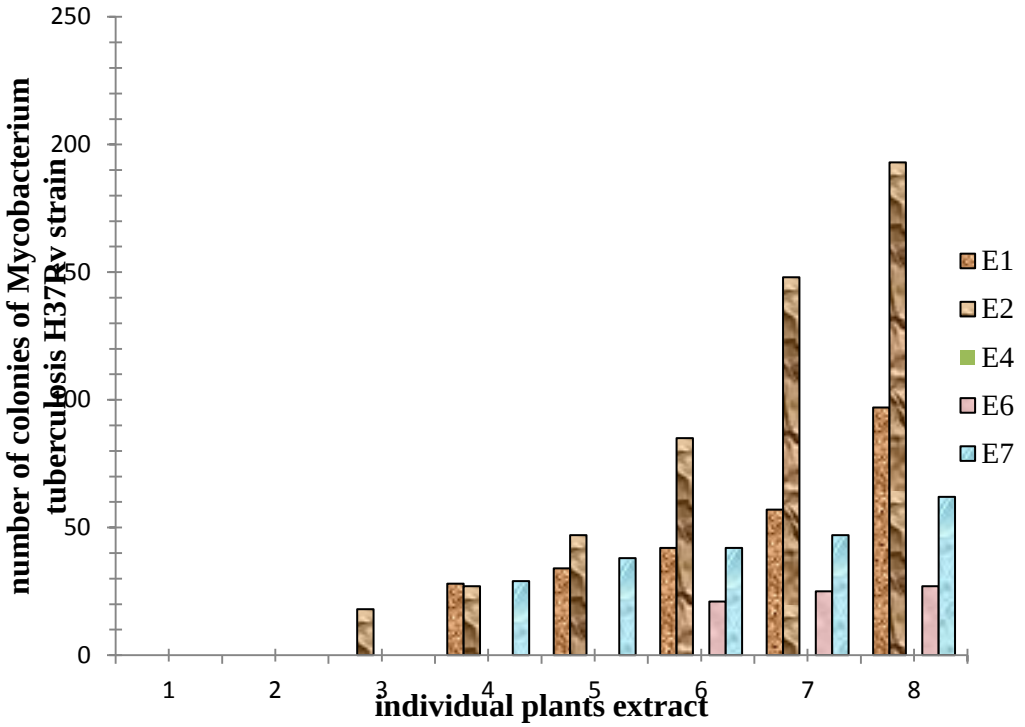

Figure 6: Susceptibility of *M. tuberculosis* H37Rv strain to the test extracts at 1.0mg/ml

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.The presence of secondary metabolites such as tannins, saponins, alkaloids and phenols, have been discussed to be causing their antibacterial activities in humans (Rojas, *et al.*, 2006; Nikitina, V.S., et al., 2007; Udobi, *et al.*, 2008). The antibacterial activities exhibited by

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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 set-up 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 been 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.

The synergistic effects of ethanol extract of ginger and garlic against Bacillus spp. and Staphylococcus aureus (Onyeagba, *et al.,* 2004). 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 to Sebiomo (2010) Zingiber officinale has the potentional of inhibiting the

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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 to Uchechi,, Ekwenye & Okorie, (2010) Tetrapleura tetraptera extracts both water ethanol 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.

The traditional herbal medicine users use all parts plant to suppress, manage, and cure diseases. 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). 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; Hostettman, & Martson, 2002).The results from this study corroborate, the importance

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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. 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).

CONCLUSION

Allium sativum exhibited very great inhibitory property by recording zero growth count throughout the eight week of culturing *Mycobacterium tuberculosis H37Rv* and *Mycobacterium bovis*. Comparing the growth *Mycobacterium tuberculosis H37Rv*and *Mycobacterium bovis* on *Allium sativum* with the first line drug, it could be observed that *Allium sativum* has high inhibition property against both strains same as isoniazid. *Lantana hispida* was able to inhibit the growth of *Mycobacterium tuberculosis H37Rv* for the first six weeks and showed growth less 10 for remaining two weeks of culturing. *Allium sativum* and *Lantana hispida* recorded an impressive inhibition against both strains and their inhibition rate was comparatively the same as isoniazid which is a first line drug for tuberculosis treatment. The rest of the plants could not record good inhibition property against *Mycobacterium bovis* and *Mycobacterium tuberculosis* throughout the culturing period. It could be concluded that *Allium sativum* and *Lantana hispida* have the potential of controlling tuberculosis and a good source for the new

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discovery of drugs to replace the existing tuberculosis drugs. The study has also revealed and validated scientifically that, there is high potential of medicinal plants which can treat tuberculosis in the shortest possible time and that much work is needed to research into the bioassay of the various phyto-constitutents of active plants especially from higher plants.

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