CHAPTER-V

ANTIMICROBIAL ACTIVITY
5.1 Anti Microbial Activity

Medicinal plants are a source of great economic value all over the world. The use of plants for medicinal purposes dates back to antiquity and has been very important in the health care delivery of every nation at one stage or another. People on all continents have been long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory (Cowan, 1999). There is evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock (Stock, 1988), which is still widely used in ethnomedicine around the world. Plants are a goldmine of novel chemicals and large numbers of modern drugs have been developed from them. There are more than 2,70,000 higher plants existing in this planet. But so far less than 10% of recorded flora has been explored phytochemically as well as clinically for various biological activities (Reddy, 2010). However, discovery of vast majority of the plant resources are still awaiting for discovery. It is estimated that among 10% of all flowering plants on Earth, which have been explored to treat various infections, only 1% have gained recognition by modern scientists (Lewis et al., 2006).

In present scenario management of plant and human diseases are generally achieved by the use of various synthetic pesticides as well as different antibiotics (Mathur et al., 1999), however indiscriminate use of these synthetic antibiotics has caused health hazards in animals and humans due to their residual toxicity and many a times management of pathogenic microbes becomes difficult due to the resistance of pathogens. Thus the large number of synthetic drugs produced by pharmaceutical industries from time to time has led to the development of resistant microorganisms that has
become a major global issue in the treatment of infectious diseases (Schinor et al., 2007). Bioactive principles isolated from plants appear to be one of the promising alternatives for the control of these antibiotic resistant plants and human pathogens.

Plants are complex storehouses of several undiscovered dynamic chemical compounds, many of which serve as part of plant defense mechanisms against invasion by micro-organisms, insects and herbivores that can provide valuable sources of natural antibacterial agents (Roosita et al., 2008; Abel et al., 2005). The active principles isolated from plants appear to be one of the important alternatives, when compared with many sub standard orthodox synthetic medicines because of their less or no side effect and better bioavailability. Plant extracts have been studied for their activities against pathogens for years and for assays to detect new and previously undiscovered antimicrobial compounds (Scazzocchio et al., 2001; Rajeswari et al., 1992). It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez et al., 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms (Davis, 1982). Therefore, there is need to search new infection-fighting strategies to control microbial infections. Many plants have been known to synthesize active
secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Reynolds, 1996; Lis-Balchin et al., 1997).

With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from antibiotics. This is because of the emergence of resistant pathogens that is beyond doubt due to the consequence of years of widespread indiscriminate use and abuse of antibiotics (Mullingen et al., 1993; Enne et al., 2001; Westh et al., 2004). Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Zgoda et al., 2001; Berghe et al., 1991).

Amongst various medicinal plants *Nerium oleander* L. (Apocyanaceae family) has been studied during the present research endeavors. It is known to contain active cardiac glycosides which are used in the treatment of cardiac abnormalities, neurological disorders, dermatitis, eczema, psoriasis, herpes, sores, abscesses, warts, corns, skin cancer, ringworm, scabies, epilepsy, abortifacients, asthma, malaria dysmenorrheal, emetics and diuretics (Henary et al., 2011; Newman, 2008; Radford et al., 1986; Duke et al., 1985; Leporatti et al., 1985; Eddouks et al., 2002; Manna et al., 2000; Zibbu et al., 2010).

Other plant chosen is ginger, the rhizome of *Zingiber officinale*, is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment for various foods and beverages. It has a long history of
medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds. The anti-inflammatory properties of ginger have been known and valued for centuries. The original discoveries of ginger’s inhibitory effects on prostaglandin biosynthesis in the early 1970s have been repeatedly confirmed. This discovery identified ginger as a herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs (Thomson et al., 2002; Grzanna et al., 2005). Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine. It is commonly used to treat various types of “stomach problems,” including motion sickness, morning sickness, colic, upset stomach, gas, diarrhea, nausea caused due to cancer drugs and nausea, loss of appetite and vomiting after surgery (American cancer society 2013; Ernst et al., 2000; Wood et al., 2000; Grontved et al., 2000; Saraswat et al., 2010; Amin et al., 2006; Afshari et al., 2007).

Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. Moreover, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al., 2002). For this reason, we have evaluated antimicrobial activity of the methanolic and ethanolic extract of *Nerium oleander* L. (leaves and root) and *Zingiber officinale* Roscoe (rhizome) against four human pathogens.

5.1.1 Material and Methods

5.1.1.1 Collection of plant material

Plants of *Nerium oleander* L. and *Zingiber Officinale* Roscoe rhizomes were collected from the Rajasthan Agricultural Research Institute, Durgapura, Jaipur Rajasthan. Specimens were compared with the voucher specimens at
Herbarium of Department of Botany, University of Rajasthan, Jaipur. Leaves and roots of *N. oleander* and rhizomes of *Z. officinale* were used for further studies.

5.1.1.2 Preparation of the extracts

To make the plant extracts, different plant parts (Leaves and root of Nerium and rhizome of ginger) were dried at room temperature and milled to a coarse powder via. mortar and pestle. Powdered material (50 gm) was soxhlet extracted with 100% methanol and ethanol for 72 hour. Extracts were evaporated in vacuum under reduced pressure. All extracts were stored at 4°C in a refrigerator until screened, their final volume was raised to a known concentration in their respective solvents before use. These extracts were further used for the detection of anti-bacterial activity.

5.1.1.3 Microorganism assayed

**Bacteria:** For all experimental pure cultures of pathogenic bacterial strains *viz.* *Escherischia coli* (Gram -ve), *Staphylococcus aureus* (Gram +ve), *Enterococcus faecalis*, *Pseudomonas aeruginosa* were maintained on nutrient agar medium at 37°C using standard procedures.

5.1.1.4 Antimicrobial assay

**Broth Dilution Method:**

The antimicrobial activity of plant extract against four strains of pathogenic bacteria *Escherichia Coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) were determined by broth dilution method (Mustafa *et al.*, 1999). Plant extracts of 1 gram/ml were taken as starting concentration in the study. 100µL of Brain Heart Infusion (BHI) broth was pipetted into the corresponding well of a sterile 96 well microtiter plate. Serial double dilutions of plant extracts were prepared in BHI broth. Bacterial suspensions from an
overnight culture were standardized to 0.5 McFarland (1.5 X 10^8 CFU mL-1). McFarland standered was made by specified amount of barium chloride (BaCl_2.2H_2O) and sulfuric acid (H_2SO_4) using an API turbidometer. A 1:20 dilution was made to give a bacterial suspension of an approximately 6 X 10^6 CFU mL-1. 10µL of the bacterial suspension was added to each well giving a final suspension of 6 x 10^6 CFU mL-1. 96 well plate was incubated at 37^0C for overnight. Next day these serial diluted samples were inoculated on Nutrient Agar and MacConkey Agar plate. Plates were further incubated at 37^0C for overnight. All experiment was run in duplicate. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that showed no visible growth in broth microdilution tray and showed mild growth when sub cultured on a suitable solid medium.

5.1.2 Results and Discussion

The findings of the present study revealed that *Nerium oleander* L. and *Zingiber officinale* Roscoe. Contain potent antimicrobial property against tested bacteria. The antimicrobial activity was evaluated by broth micro dilution method using four strains of pathogenic bacteria (*Escherichia Coli ATCC 25922, Pseudomonas aeruginosa ATCC27853, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212*). These extracts exhibited strong antimicrobial activity. The results obtained in the broth dilution assay regarding the MIC ranges of all the four bacterial strains were concentration dependent as shown in (Table 5.1 and Table 5.2).

Amongst *N. oleander* ethanolic and methanolic extracts maximum activity was attributed against *P. aeruginosa* and *S. aureus* (1.75±0.092, 1.75±0.092 and 2.00±0.01, 2.00±0.02 respectively). While moderate activity against *E. coli* (2.00±0.04 and 2.50±0.02 mg/ml) and least activity against *E. faecalis* (2.5±0.06 and 3.50±0.01).Thus *N. oleander* ethanolic extract and *Z. officinale* methanolic extract showed maximum activity against *P. aeruginosa*
Fig. A-B: Antimicrobial activity of various extracts of *Nerium oleander* L. and *Zingiber officinale* Roscoe. against *E. coli* and *P. aeruginosa*

Fig. A: *P. aeruginosa*
Fig. B: *E. coli*

Abbreviations:
NM - *Nerium Oleander* L. Methanolic Extract
NE - *Nerium Oleander* L. Ethanolic Extract
ZM - *Zingiber Officinale* Roscoe. Methanolic Extract
ZE - *Zingiber Officinale* Roscoe. Ethanolic Extract
Fig. A-B: Antimicrobial activity of various extracts of *Nerium oleander* L. and *Zingiber officinale* Roscoe. against *S. aureus* and *E. faecalis*

Fig. A: *S. aureus*
Fig. B: *E. faecalis*

Abbreviations:
NM - *Nerium Oleander* L. Methanolic Extract
NE - *Nerium Oleander* L. Ethanolic Extract
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ZE - *Zingiber Officinale* Roscoe. Ethanolic Extract
Table 5.1: Antimicrobial screening of *Zingiber officinale* extracts

<table>
<thead>
<tr>
<th>Test microorganism</th>
<th>Extract</th>
<th>MIC range (mg/ml)</th>
<th>MIC range (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanolic</td>
<td>2.0±0.04</td>
<td>3.50±0.02</td>
</tr>
<tr>
<td></td>
<td>Methanolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. Coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>2.0±0.01</td>
<td>1.75±0.09</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>2.0±0.02</td>
<td>1.75±0.08</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td></td>
<td>2.0±0.06</td>
<td>3.50±0.01</td>
</tr>
</tbody>
</table>

Table 5.2: Antimicrobial screening of *Nerium oleander* L. extracts

<table>
<thead>
<tr>
<th>Test microorganism</th>
<th>Extract</th>
<th>MIC range (mg/ml)</th>
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</tr>
</thead>
<tbody>
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<td></td>
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<td>3.50±0.01</td>
</tr>
</tbody>
</table>
and *S. aureus*, while least activity was observed against *E. faecalis*. In conclusion, methanol extract of *Z. officinale* and ethanol extract of *N. oleander* were found to have more promising antibacterial activity in comparison to their ethanol and methanol extracts, respectively.

In case of both extracts of *Zingiber officinale*, ginger methanolic extract showed maximum activity against *P. aeruginosa* and *S. aureus* (1.75±0.09 mg/ml). Whereas the activity was recorded minimum against *E. coli* and *E. faecalis* (3.50±0.02 mg/ml). Moderate activity was observed with rhizome ethanolic extracts against all the four pathogens viz., *E. coli* (2.00±0.04 mg/ml), *S. aureus* (2.00±0.02 mg/ml), *E. faecalis* (2.00±0.06 mg/ml) and *P. aeruginosa* (2.00±0.01 mg/ml). Therefore, it can be concluded that *Z. officinale* methanolic extract possess the great antimicrobial activity against *P. aeruginosa* and *S. aureus*.

Earlier, many researchers have reported antimicrobial activity of *N. oleander* and *N. indicum* by various methods e.g. Disc diffusion technique, Serial dilution technique and Ditch plate technique (Mostaqul *et al.*, 1999; Reddy *et al.*, 2010; Ai-min *et al.*, 2008; Naqvi *et al.*, 1994; Chauhan *et al.*, 2013; Hussain *et al.*, 2004; Ali *et al.*, 2010; Derwic *et al.*, 2010; Namian *et al.*, 2013). Rajendran *et al.*, (2011) screened antibacterial and antifungal activity of *N. oleander* against different organisms and found it highly active against *Staphylococcus aurcus*, *Staphylococcus albus* and klebsilla species. In 2007 Bhuvaneshwari and colleagues analysed that growth of *Staphylococcus aurcus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* is controlled by *N. oleander*. Moreover Doijad *et al.*, (2013) concluded that that leaves of *N. indicum* possess higher antibacterial activity against Gram positive microorganism *Bacillus subtilis* but negligible activity against Gram negative microorganism *E-coli*. Similarly Sawi *et al.*, (2010) investigated antibacterial properties of the crude and pure extracts of *N. oleander* against various gram
positive and gram negative bacteria and found crude extract of *N. oleander* to be more effective than purified compound.

Several *in vitro* studies have shown that ginger extract and its active constituents inhibit growth of several pathogens e.g. *Escherichia coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Proteus* sp., *Staphylococci* sp., *Streptococci* sp. and *Salmonella* sp., (Sebiomo *et al.*, 2011; Gao *et al.*, 2010; Yu *et al.*, 2009; Malu *et al.*, 2008; Akoachere *et al.*, 2002; Cwikla *et al.*, 2010; Singh *et al.*, 2008; Betoni *et al.*, 2006; Nostro *et al.*, 2006; Mahony *et al.*, 2005; Konning *et al.*, 2004; Samy, 2005; Thongson *et al.*, 2005; Park *et al.*, 2008; Maekawa *et al.*, 2013; ). In 2012 Gull and colleagues determined MIC values for *E. Coli* (0.08 mg/ml), *P. aeruginosa* (0.4 mg/ml), *B. subtilis* (0.3 mg/ml) and *S. epidermidis* (0.05 mg/ml) with ginger methanol extract while MIC values for *S. aureus* (0.3 mg/ml), *K. pneumonia* (0.05 mg/ml) and *S. typhi* (0.08 mg/ml) with ginger ethanol extract. They concluded that Gram positive bacteria were more sensitive than Gram negative bacteria. Further they concluded that ginger alcoholic extracts are more effective than aqueous extracts (Gull *et al.*, 2012). Various other scientists also determined antimicrobial activity of *Zingiber officinale* using disc diffusion method. Karuppiah *et al.*, (2012) investigated that ginger rhizome ethanolic extracts demonstrated antibacterial activity against five clinical isolates with zone of growth inhibition ranging from 4 mm to 16 mm. The maximum zone of inhibition was showed against Bacillus sp. (16.55 mm) followed by *E. coli* (15.50 mm) and *P. aeruginosa* (14.45 mm). The minimum diameter of zone of growth inhibition was recorded against Klebsiella sp. (5 mm) and Enterobacter sp. (4 mm). Similar results were obtained by Belllik *et al.*, (2014), they determined that *E. coli* was the most susceptible to the action of oleoresin with an inhibition zone diameter of 40 mm followed by *B. subtilis* (31 mm) and *S. aureus* (20 mm), whereas ginger essential oil was more active on *S. aureus* (30 mm). The antifungal activity of ginger oleoresin and essential oil was effective in inhibiting *Penicillium* spp. at the concentrations of 2 mg/mL and
869.2 mg/mL, respectively. These result were in accordance with the findings of other scientist (Chandarana et al., 2005; Onyeagba et al., 2004; De-Souza et al., 2005; Park et al., 2008; Kaushik et al., 2011; Takahashi et al., 2011; Sasidharan et al., 2010; Singh et al., 2008).

However, ginger has been used widely as herbal medicine and its gingerol-related components have been reported to possess antimicrobial and antifungal properties, as well as several other pharmaceutical properties (Park et al., 2008). Manjunatha et al., (2013) explored antimicrobial activity of Zingerone and its derivatives against both Gram-positive and Gram-negative bacteria. In 2013 Kubra and colleagues revealed a potential antifungal agent Dehydrozingerone named dehydroderivative of zingerone. Park et al., (2008) reported 10-gingerol and 12-gingerol as effective growth inhibitors of oral pathogens at a MIC range of 6-30 microg/mL. Wang et al., (2010) also determined antimicrobial activity of 6-dehydrogingerdione, 10-gingerol, 6-shogaol and 6-gingerol.

5.2 Anti Mycobacterial Activity

Tuberculosis, or TB, is an infectious bacterial disease caused by Mycobacterium tuberculosis, which most commonly affects lungs (Affloabi et al., 2007; Ducati et al., 2006; Zumla et al., 1998). It is transmitted from person to person via droplets from throat and lungs of patients with active respiratory TB disease. Presently one third of the world population is currently infected with the TB bacillus and 8.7 million new cases occur annually. Approximately 1.1 million deaths from the disease per year, equivalent to 125 cases per 100 000 population. The highest prevalence of cases is in South-East Asia (SEA) region. The Region carries about 40% of the global burden of tuberculosis with an estimated 5 million prevalent and about 3.5 million incident cases in 2010 among them India alone accounting for more than 25% of the world’s incident cases (WHO 2013). Many anti-TB
drugs are available to treat TB e.g. Streptomycin (STR), Rifampicin (RIF), Isoniazid (INH), Ethambutol (EMB), Pyrazinamide (PZA) etc. Inspite of all these drugs, emergence of multi drug resistance (MDR) and extensively drug resistant TB (XDR-TB) in tuberculosis is a matter of great concern for TB control programs. There were an estimated 630 000 cases of MDR-TB (range, 460 000–790 000) among the world’s 12 million prevalent cases of TB. Globally, 3.7% (2.1–5.2%) of new cases and 20% (13–26%) of previously treated cases are estimated to have MDR-TB (WHO 2013).

The rapid spread of MDR-TB and XDR-TB strains around the world have shown the urgent need for the development of new TB drugs to shorten the duration of treatment and fight against MDR-TB and XDR-TB strains. Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties (Heinrich et al., 2001; Tripathi et al., 2005; Gu et al., 2004). Among them one of the traditionally used medicinal herbs is *Zingiber officinale* Roscoe. which is commonly known as Ginger. It contains potent antioxidant (Kinghorn et al., 2009), anti-inflammatory (Penna et al., 2003), anti-lipid (Kadnur et al., 2005), anti-diabetic (Islam et al., 2008; Kato et al., 2006) and anti-tumor activities (Kim et al., 2008). Similarly *Nerium oleander* L. (common name: Kaner) belonging to the family Apocynaceae is widely distributed in temperate regions throughout the world. It is known to contain active cardiac glycosides which are used in the treatment of cardiac abnormalities, neurological disorders, dermatitis, eczema, asthma and diuretics (Adome et al., 2003; Sudha et al., 2011; Manna et al., 2000). The present study is designed to explore the primalary antimycobacterial potential of two drought resistance plants: *Z. officinale* and *N. oleander*. 
5.2.1 Materials and Methods

5.2.1.1 Test micro-organisms

The test mycobacterium strain, *Mycobacteria tuberculosis* (H$_{37}$ RV) strain (ATCC 27294) was sub-cultured on Lowenstein Jensen (LJ) slants and incubated at 37°C for 2-6 weeks using standard procedures, optimum growth was further used within 14 days.

5.2.1.2 Nitrate Reduction Assay (NRA)

Culture and Drug sensitivity of MTB from clinical material requires 10-12 weeks time by LJ which is “GOLD STANDARD”. Therefore NRA, Resazurin Microplate Assay (REMA), Tetrazolium microplate assay (TEMA) and Microplate Alamar Blue Assay (MABA) could be the good alternatives due to their rapidity and reproducibility with their cost effectiveness (Todd *et al.*, 2007).

Amongst above mentioned various techniques, NRA is rapid, inexpensive and simple, which could also be established in remote areas of low-resource countries. Therefore, for the detection of anti-TB activity of plant samples, NRA was performed as described by Angeby *et al.*, in 2002 with the slight modifications. The *M.tuberculosis* H$_{37}$Rv strain (ATCC 27294) was cultured on Lowestein-Jensenn medium at 37°C until log phase growth; then a cell suspension was prepared at a concentration of about 1 McFarland 3x10$^7$ CFU/ml and further diluted 1:20 in Middle brook 7H9 (Becton Dickinson and Co., Sparks MD, USA) medium which was supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase).

100 µl of sterile medium was introduced into well 2-11 row B-G of a sterile 96 well micro titer plate to each well. 100 µl of plant extract was added into 5th well of each row. Serial two fold dilution of plant extracts were prepared directly into microtiter plate.100 µl of inoculum was added into each media containing well.
200 µl of sterile water was added to all outer periphery wells to avoid evaporation during incubation. The plate was covered with its lid and sealed with parafilm and then incubated at 37° C under a normal atmosphere. After 7 days of incubation, 0.5 ml freshly prepared Griess reagent mixture (one part 50% conc. HCl, 2 parts 0.2% sulphanilamide and 2 parts 0.1% N-1 naphthylethylenediamine dihydrochloride) was added to the control. The result was classified as negative, no color change or positive depending on the color change, from 1+ (pink) to 4+ (deep red to violet).

If the result in the control (drug free well) had at least 2+ positive, the drug containing well would develop color, otherwise the whole plate was reincubated and the procedure was repeated at day 10, day 14 and finally at day 21. A strain was considered resistant, if the extract containing well produced a color change that was similar to or more intense than that in the control. A strain was considered susceptible if there was no color change in extract containing well or less than that in control well.

5.2.2 Results & Discussion

NRA tests are rapid and inexpensive and easier to implement. The results showed that methanol and ethanol extracts of both the plant species possessed antimycobacterial activity (Table 5.3). In the present study methanolic extract of both the plants showed higher antimycobacterial activity than their ethanolic extracts.

Various scientist worked on anti- mycobacterial activity using the plant extracts e.g eleven traditional medicinal plants of Colombia were studied by Bueno-Sánchez et al., (2009) and they observed that essential oils from Achyrocline alata and Swinglea glutinosa are the most active with MIC of 62.5±0.1 and 100±36 µg ml-1, respectively. Pavan et al., (2009) evaluated 37 plant species from Brazil against MTB by the use of Microplate Alamar Blue
Figure A: Anti Mycobacterial tuberculosis activity of *Zingiber officinale* Roscoe. methanolic and ethanolic extract

Figure B: Anti Mycobacterial tuberculosis activity of *Nerium oleander* L. methanolic and ethanolic extract
### Table 5.3: Preliminary anti-tuberculosis screening of both Plant species

<table>
<thead>
<tr>
<th>Plants Extracts</th>
<th>Concentration Range Measured</th>
<th>Growth Inhibition Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nerium oleander</em> L. (Leaves) EtOH</td>
<td>44.64 – 0.087 mg/ml</td>
<td>1.37 – 2.75 mg/ml</td>
</tr>
<tr>
<td><em>Nerium oleander</em> L. (Leaves) MtOH</td>
<td>24.85 – 0.048 mg/ml</td>
<td>1.55 – 3.10 mg/ml</td>
</tr>
<tr>
<td><em>Zinger officinale</em> Roscoe (Rhizome) EtOH</td>
<td>33.57 – 0.065 mg/ml</td>
<td>1.05 – 4.20 mg/ml</td>
</tr>
<tr>
<td><em>Zinger officinale</em> Roscoe (Rhizome) MtOH</td>
<td>28.15 – 0.054 mg/ml</td>
<td>1.75 – 3.51 mg/ml</td>
</tr>
</tbody>
</table>
Assay (MABA). Crude extracts from sixteen plants showed MIC value of \( \leq 125 \, \mu g/mL \) and three 31.2 \( \mu g/mL \). Few plant extracts were tested against four strains of Mycobacteria namely; *Mycobacterium tuberculosis* (MtB), *M. Kansasi* (Mk), *M. fortuitum* (Mf), and *M. smegmatis* (Ms) using BACTEC MGIT 960 system by Mariita et al (2010). They observed that extracts of *Scadoxus multiflorus* and *Acacia nilotica* showed strong antimycobacterial activity. Akihisa et al., (2005) isolated triterpenoids from the non-saponifiable lipid fraction of the flower extract of chrysanthemum (*Chrysanthemum morifolium*) and tested them for their antitubercular activity against *Mycobacterium tuberculosis* strain H37Rv using MABA. Crude methanolic extracts of the aerial parts of the two *Laggera* species (*Laggera pterodonta* (DC.) Sch. Bip. and *Laggera aurita* (Linn f.) DC.) of the family Asteraceae (Compositea), found in Nigeria were screened against *Mycobacterial bovis* (BCG strains). The two extracts were found to be active at MIC of 625 \( \mu g/ml \) (Egharevba et al., 2010). Many other scientists also evaluated antimycobacterial potential of various medicinally important traditional plants (Balachandran et al., 2012; de Oliveira et al., 2012; Polatoğlu et al., 2013; Panda et al., 2014; Fomogne-Fodjo et al., 2014; Lirio et al., 2014; Bussey et al., 2014; Naik et al., 2014; Gupta et al., 2014).

**Conclusion**

In conclusion, we observed that both plants (*N. oleander* and *Z. officinale*) had antimicrobial activity. However, we evaluated the antimicrobial potential of only crude alcoholic extracts. Moreover to understand the proper antimicrobial potential of these two plants, it is necessary to isolate and identify the active constituents of both plant extracts.