

## **ANTIMYCOBACTERIAL ACTIVITY OF ISOLATED COMPOUNDS FROM *LIPPIA JAVANICA* AND *HOSLUNDIA OPPOSITA***

### **Abstract**

Eight compounds isolated from *Lippia javanica* and three compounds, from *Hoslundia opposita* were tested against *Mycobacterium tuberculosis* at concentrations of 200, 100, 50 and 25 µg/ml. Compound “6-Methoxyluteolin 4'-methyl ether” isolated from *L. javanica* exhibited a minimum inhibitory concentration (MIC) of 200 µg/ml against *M. tuberculosis*. Of all the compounds tested against a drug-sensitive strain of *M. tuberculosis*, euscaphic acid was found to show the best activity exhibiting an MIC of 50 µg/ml against this strain. The remaining compounds were found to be inactive at the highest concentrations tested.

### **7.1 Introduction**

Tuberculosis is a bacterial disease caused mainly by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *Mycobacterium tuberculosis* was isolated by Robert Koch in 1882. *Mycobacterium bovis* is responsible for tuberculosis in domestic or wild cattle. *M. bovis* infections are uncommon in most countries today. In the past, this infection was often transmitted through the oral route by drinking milk from infected cows

(Porter & McAdam, 1994). Virtually all new infections with *M. tuberculosis* are acquired via airborne transmission. The sources of infections are persons with tuberculosis of the lung or larynx who are coughing. 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 periods of time unless they are removed by ventilation, filtration or ultraviolet irradiation.

Tuberculosis is an ancient disease. It was present in Egypt from early dynastic times, perhaps as early as 3700 BC (Morse *et al.*, 1964). Manchester (1984) has reviewed evidence that suggests that human tuberculosis may have evolved during the Neolithic period (seventh and sixth millennia BC) at which time population increases and cattle domestication occurred in Europe and the eastern Mediterranean. Tuberculosis was well recognized by the time of Hippocrates (c. 460-377 BC) who gave an excellent clinical description of the disease (Hippocrates, 1939). In India, the medical Luminary Susruta (c.500 AD) mentioned the disease in his writings (Pierry & Roshem, 1931). WHO (2000) estimates that between the years 2000 and 2020 nearly one billion people will die from the disease. The greater majority of the world's population, and thus the majority of infected persons, reside in developing countries (Snider *et al.*, 2005).

The number of cases worldwide is rapidly increasing due to the appearance of single-drug-resistance (SDR) and multidrug-resistance (MDR) of strains of *M. tuberculosis* which are insensitive to one or more the first-line anti-TB drugs (isoniazid [INH], rifampin, ethambutol, streptomycin and pyrazinamide (Telzak *et al.*, 1995) and also

due to an increase in patients with immunodeficiency virus (HIV) infection, which has further exacerbated the problem (Zumla & Grange, 1998). The emergence of strains of *Mycobacterium tuberculosis* resistant to existing drugs has focussed attention on the urgent need for discovery and development of new antimycobacterial agents. Action must be taken now to avert this global health disaster.

There is a need for more intense efforts in the discovery of new specific drugs from natural and synthetic sources. There are reports on inhibition of mycobacteria by medicinal plants. The compound allicin from *Allium sativum* was found to be as potent as some of the standard antitubercular drugs such as streptomycin, isoniazid, ethambutol and rifampin (Jain, 1994). Allicin, prepared from the ethanolic extract inhibited the growth of *Mycobacterium tuberculosis* H37Rv and *M. tuberculosis* TRC-C1193 that is completely resistant to isoniazid. The MIC was 70 µg/ml for both the organism (Indian Council of Medical Research, 2004). Lall (2000) reported antitubercular activity of naphthoquinone 7-methyljuglone isolated from *Euclea natalensis*. The compound was tested against a drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* and the minimal inhibitory concentration (MIC) were found to be 50 µg/ml for both the strains of *M. tuberculosis*. This may mean that there should be an abundance of antitubercular drugs remaining to be discovered in plants.

This chapter focuses on the antimycobacterial activity of compounds isolated from *Lippia javanica* and *Hoslundia opposita*.

## **7.2 Materials and methods**

### **7.2.1 Bioassay on *Mycobacterium tuberculosis***

Anti-TB activity of compounds against *M. tuberculosis* H37Rv was determined using the radiometric respiratory technique with the BACTEC apparatus as described in chapter 2 of this thesis. The nine compounds (3 isolated from *H. opposita*) and 6 isolated from *L. javanica* were dissolved at 20 mg/ml in 1 % DMSO. Subsequent dilutions were done in DMSO and added to 4 ml of BACTEC 12B broth to achieve the desired final concentrations of 200, 100, 50 and 25 µg/ml together with PANTA (Becton Dickinson & Company), an antimicrobial supplement. The BACTEC drugs susceptibility testing was also done for the two primary drugs streptomycin and ethambutol at concentrations of 6 and 7.5µg/ml respectively against the H37Rv sensitive strain. Preparation of bacterial cultures and the testing procedures were the same as described in chapter 2. All tests were done in triplicate.

## **7.3 Results and discussion**

Results were interpreted on day 6 or 7 when the control vials containing the 1:100 dilution of the inoculum reached a GI value of 30 or more (Table 7.1). Among the nine compounds tested, the MIC of jacarandic acid or euscaphic acid, isolated from *Hoslundia opposita* was found to be 50µg/ml against the H37Rv. strain. This indicated that the strain is partially susceptible to the compound at a low

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concentration of 50µg/ml. The MIC of the terpene compound, 6-Methoxyluteolin 4'-methyl ether, isolated from *L. javanica* was found to be 200 µg/ml. The remaining compounds were inactive. Not much information is available in the literature about the antimycobacterium activities of natural triterpenes, however similar activities were found observed in ursane triterpenes (Mujovo *et al.*, 2008).

**Table 7.1** Anti-tuberculosis activity of compounds found in *L. javanica* and *H. opposita*

Compounds tested	MIC <sup>a</sup> µg/ml	ΔG values of compounds µg/ml
<b>From <i>L. javanica</i></b>		
6-Methoxyluteolin 4'-methyl ether	200	12 ± 2
Cirsimaritin	na <sup>b</sup>	> 200
6-Methoxyluteolin 3',4',7-trimethyl ether	na <sup>b</sup>	> 200
Apigenin	na <sup>b</sup>	> 200
1-(3,3-dimethoxyranyl)-3-methyl- (2 <i>E</i> )	na <sup>b</sup>	> 200
Pipertinone	na <sup>b</sup>	> 200
β-sistosterol	na <sup>b</sup>	> 200
4-Ethyl-nonacosane (C <sub>31</sub> H <sub>64</sub> )	na <sup>b</sup>	> 200
<b>From <i>H. opposita</i></b>		
Digitoxypyranosyltectochoysin or Hoslunddiol	na <sup>b</sup>	> 200
5,7-dimethoxy-6-metylflavone	na <sup>b</sup>	> 200
Jacarandic acid or euscaphic acid	50	6 ± 0.0

ΔG Control: 26.5 ± 3.5,

<sup>a</sup> minimal inhibitory concentration, ΔG values are means ± standard deviation

<sup>b</sup> not active at the highest concentration tested

## 7.4 Conclusion

Of all the compounds tested against a drug-sensitive strain of *M. tuberculosis*, euscaphic acid was found to show the best activity exhibiting an MIC of 50 µg/ml

against this strain. The compound deserves further investigation in order to explore its potential as an antimicrobial agent.

## 7.5 References

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## ANTI-HIV ACTIVITY OF ISOLATED COMPOUNDS FROM *LIPPIA JAVANICA* AND *HOSLUNDIA OPPOSITA*

### Abstract

The discovery of medicinal agents capable of specifically inhibiting human immunodeficiency virus (HIV) is urgently needed due to its globally widespread infection. In this study, compounds isolated from *Lippia javanica* and *Hoslundia opposita* were investigated for their ability to inhibit HIV-1 Reverse transcriptase activity *in vitro* using a non-radioactive assay. Two compounds “1-(3,3-dimethoxyiranyl)-3-methyl-penta-2, 4-dien-1-one” and “Pipertinone” from *L. javanica* demonstrated inhibitory activity against the enzyme by 90 and 53 %, respectively at 100 µg/ml. One compound “5, 7-dimethoxy-6-methylflavone” isolated from *H. opposita* was shown to have 52 % inhibition at 100 µg/ml.

### 8.1 Introduction

Acquired immunodeficiency syndrome (AIDS) is a pandemic immunosuppressive disease which results in life-threatening opportunistic infections and malignancies.

Human immunodeficiency virus (HIV) requires three key enzymes for viral replication inside a host cell, Reverse transcriptase, protease and integrase. Reverse transcriptase is one of the main targets for inhibiting the reproduction of HIV. This enzyme is responsible for transcription of viral RNA into a DNA, which is later,



integrated into the host cell and carries the information for the synthesis of new viral particles. Inhibition of the HIV of HIV-1 RT, besides the later discovery HIV protease and integrase inhibition was the first therapeutic approach successfully applied in prolonging the life of infected patients (Barre-Sinoussi, 1996). Searching for novel inhibitors of the HIV replication cycle is the main interests of numerous investigators and enormous efforts have been dedicated to finding promising lead compounds, both synthetic and natural (De Clercq, 1995). Inhibition of retroviral RTs by plant derived compounds has previously been described. Since a retrovirus (HIV) has been clearly identified as the primary cause of AIDS, many compounds of plant origin have been evaluated for their inhibitory effects on HIV replication (Vlietinck *et al.*, 1998, Ng and Huang, 1997).

## **8.2 Materials and Methods**

### **8.2.1 HIV-1 RT assay**

The assay was performed as described in chapter 3, but each compound was tested at 100 µg/ml .

## **8.3 Results and discussion**

The standard Reverse transcriptase assay is a specific, sensitive, simple and reliable method for discovery potential agents that inhibit HIV-1 and HIV-2 RT from natural sources. Evaluation of all the isolated compounds from *L. javanica* and *H. opposita*

against HIV RT showed that two compounds “1-(3,3-dimethyloxiranyl)-3-methylpenta-2, 4-dien-1-one” and “Pipertinone” from *L. javanica* demonstrated inhibitory activity against the enzyme by 90 and 53 %, respectively at 100 µg/ml. One compound “5, 7-dimethoxy-6-methylflavone” isolated from *H. opposita* was shown to have 52 % inhibition at 100 µg/ml (Table 1). Little is known about the HIV RT activity of monoterpenes in literature, however, the results indicated that compound “1-(3,3-dimethyloxiranyl)-3-methylpenta-2, 4-dien-1-one” could be of interest as a template in drug discovery research due to the higher activity rather than the other compounds isolated from both plants. There are no previous reports of the anti- HIV activity of this compound.

Flavonoids are widely distributed in nature and were found to be active against viruses HSV-1, HSV-2, rotavirus, and even against HIV (Vlitsinck *et al.*, 1998; Harborne *et al.* 1975). In HIV, their activity was related to a direct effect on the virus or the enzymes responsible for its replication (HIV-1 Reverse transcriptase or HIV-1 integrase). A flavone from *H. opposita* showed considerable inhibition against RT similar to the reports on 3-methoxyflavones. 3-Methoxyflavones, and synthetic derivatives thereof, have proven to be promising leads for developments antirhinovirus drugs (Ishitsuka *et al.*, 1982, De Meyer *et al.*, 1990). 3-methoxyflavones interfere with an early stage in the viral RNA synthesis (Lopez Pila *et al.*, 1989; Castrillo *et al.*, 1986).

**Table 8.1 Anti- HIV RT activity of compounds *L. javanica* and *H. opposita***

Compounds		% inhibition <sup>a</sup>
<b>From <i>L. javanica</i></b>		
1	4-ethyl-nonacosane	2.00 ± 0.2
2	(E)-2(3)-Tagetenone epoxide	91.00 ± 0.04
3	Myrcenone	0.60± 0.01
4	Piperitenone	53.00± 0.01
5	Apigenin	- 19.00± 0.03
6	Cirsimaritin	12.00± 0.0
7	6-Methoxyluteolin 4-methyl ether	0.70 0±.01
8	6-Methoxyluteolin 3,4,7-trimethyl ether	17.00±0.02
<b>From <i>H. opposita</i></b>		
9	5, 7- dimethoxy-6-methylflavone	52.00 ± 0.01
10	Hoslunddiol	15.00 ± 0.01
11	Jacarandic acid or euscaphic acid	3.00 ± 0.0
Adriamycin (Positive control)		96.00± 0.2

Note:<sup>a</sup>Percentage inhibition are average SD.

## 8.4 Conclusion

Two compounds (E)-2(3)-Tagetenone epoxide and “Pipertinone” from *L. javanica* demonstrated inhibitory activity against the enzyme by 90 and 53 %, respectively. One compound “5, 7-dimethoxy-6-methylflavone” isolated from *H. opposita* was shown to have 52 % inhibition. The three compounds would be interesting for further investigation.

## **8.5 References**

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## **CYTOTOXICITY OF CRUDE EXTRACTS AND THE ISOLATED COMPOUNDS FROM *LIPPIA JAVANICA* AND *HOSLUNDIA OPPOSITA***

### **Abstract**

Studies on the cytotoxicity of plant extracts are useful to evaluate the toxicological risks, The cytotoxicity tests are essential before the compounds can be considered for their impact in drug discovery. Plant extracts of *Lippia javanica*, *Hoslundia opposita* and three isolated compounds which showed promising activity in anti-HIV and antimycobacterial bioassay were evaluated for cytotoxicity against monkey kidney vero cell-lines. The compound “5,7-dimethoxy-6-methylflavone exhibited fifty percent inhibitory concentration (IC<sub>50</sub>) of 2.73 µg/ml. The IC<sub>50</sub> values of crude extracts of *Hoslundia opposita* and *Lippia javanica* were found to be 116.8 ± 6.16 µg/ml and 29.41 ± 7.845 µg/ml respectively. The other isolated compounds exhibited the following IC<sub>50</sub> values: piperitenone IC<sub>50</sub> >200, 1-(3, 3-dimethoxiranyl)-3-methyl- (2*E*), 13.96 ± 5.144, jacarandic acid or euscaphic acid IC<sub>50</sub> 19.21 ± 4.520 µg/ml.

### **9.1 Introduction**

Cytotoxicity is simply the cell-killing property of a chemical compound (such as food, cosmetics, or pharmaceuticals) or a mediator cell (such as a cytotoxic T cell),

independent from the mechanisms of death (Roche, 2004). There are various methods used for the determination of in vitro determination of cytotoxicity; such as brine shrimp, lactate dehydrogenase (LDH) assay and colorimetric assays. In this study an attempt was made to determine the cytotoxicity of crude extracts and bioactive compounds isolated from *Lippia javanica* and *Hoslundia opposita* using the colorimetric assay based on the tetrazolium reagent 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl] 2H-tetrazolium hydroxide (XTT) (Williams *et al.*, 2003).

The XTT tetrazolium salt differs from the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in that it produces a water-soluble formazan (Paull *et al.*, 1988). The formazan dye formed is soluble in aqueous solutions and is directly quantified using scanning multiwell spectrophotometer (ELISA reader). The XTT based method was used in this study because it is reliable, straightforward, efficient and inexpensive way of determining cytotoxic properties in crude biological materials and purified chemical substances.

## 9.2 Materials and Methods

Cytotoxic test of crude/pure compounds were carried out using Vero African Green monkey cell line (Terasima and Yasukawa, 1988). The microtitre plate with Vero cells were used following the method of Zheng *et al.* (2001).

### **9.2.1 Cell culture**

Vero cells were cultured in minimal essential medium (Eagle) containing 1.5 g/L sodium bicarbonate, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 10 µg/ml penicillin, 10 µg/ml streptomycin, and 0.25 µg/ml fungizone, and 10% foetal bovine serum at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. Cells were subcultured in a 1:6 ratio every second to third day after trypsinization of confluent cultures.

### **9.2.2 Preparation of cells for cytotoxicity screen**

On day 0, confluent cultures were trypsinized and diluted in complete MEM to a concentration of  $1 \times 10^5$  cells/ml. In the outer wells of a 96-well plate, 200µl of medium was dispensed. All inner wells received 100µl ( $1 \times 10^4$  cells) of the cell suspension (Figure 9.1). The plate was incubated overnight at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>.

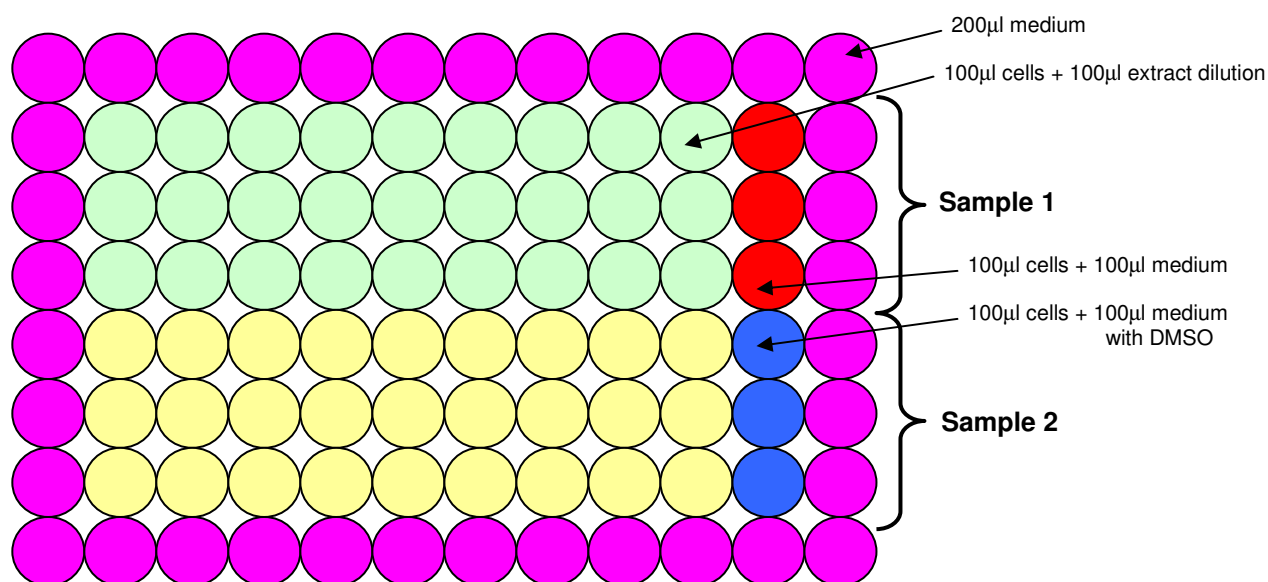
### **9.2.3 Preparation of crude extracts and pure compounds**

On day 1, stock solutions of crude extracts/pure compounds were prepared in DMSO at 20 mg/ml. Stock solutions of crude extracts were diluted 50 times in complete medium to 400 µg/ml. This was then serially diluted to obtain eight different concentrations of the

crude extracts (3.13, 6.25, 12.50, 25, 50, 100, 200, 400  $\mu\text{g/ml}$ ). Stock solutions of pure compounds were diluted 200 times in complete medium to 100  $\mu\text{g/ml}$ . This was then serially diluted to obtain eight different concentrations of the pure compounds (0.78, 1.56, 3.13, 6.25, 12.50, 25, 50, 100  $\mu\text{g/ml}$ ).

#### 9.2.4 XTT assay

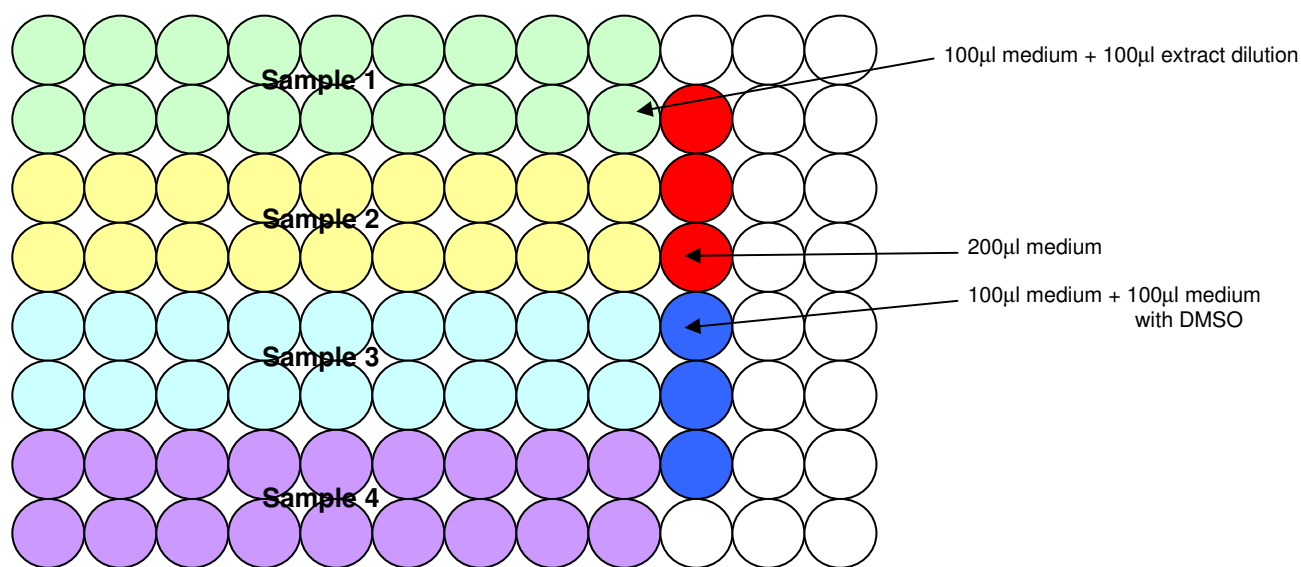
On day 1, 100  $\mu\text{l}$  of each crude extracts of *Lippia javanica* and *Hoslundia opposita*/pure compound dilution were dispensed into cell-containing wells of the sample plate in triplicate, Figure 9.2. The final concentrations of crude extracts and pure compounds in the wells were 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25, 50  $\mu\text{g/ml}$ . Control wells received a final concentration of 1% (for crude extracts) or 0,25% (for pure compounds) DMSO in complete medium.



**Figure 9.1 (a)** Assay in 96-well Sample plate



The reference plate was also prepared that contained 100  $\mu$ l of medium and 100  $\mu$ l of diluted extract/compound, in duplicate, Figure 9.3. Plates were then incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> for another 3 days. On day 3, 50 $\mu$ l of XTT reagent was added to the wells and incubation commenced for 1-4 hrs. The positive drug (Zearalenone) at final concentration of 1.25 $\mu$ g/ml was included. After incubation the absorbance of the colour complex was spectrophotometrically quantified using an ELISA plate reader, which measures the optical density at 450nm with a reference wavelength of 690nm. The 'GraphPad Prism 4' statistical program was used to analyse the fifty percent inhibitory concentration (IC<sub>50</sub>) values.



**Figure 9.1(b)** Assay in 96-well Reference plate

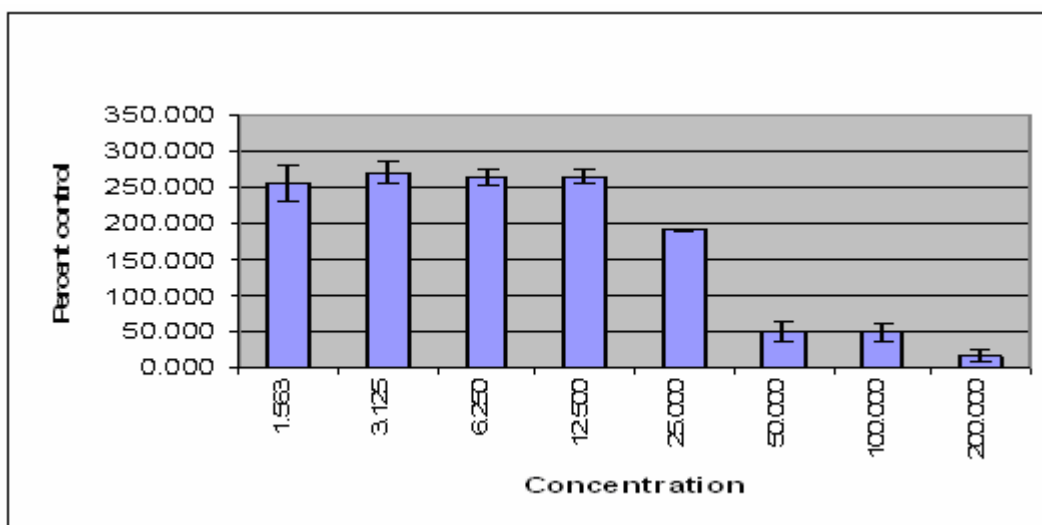
### Calculations

The 650 nm reference wavelength values were subtracted from their corresponding 450 nm wavelength values. Reference plate values were then subtracted from their corresponding sample plate values. Cell viabilities (and therefore toxicities) were assessed by determining the ratio of the sample values to the control values:

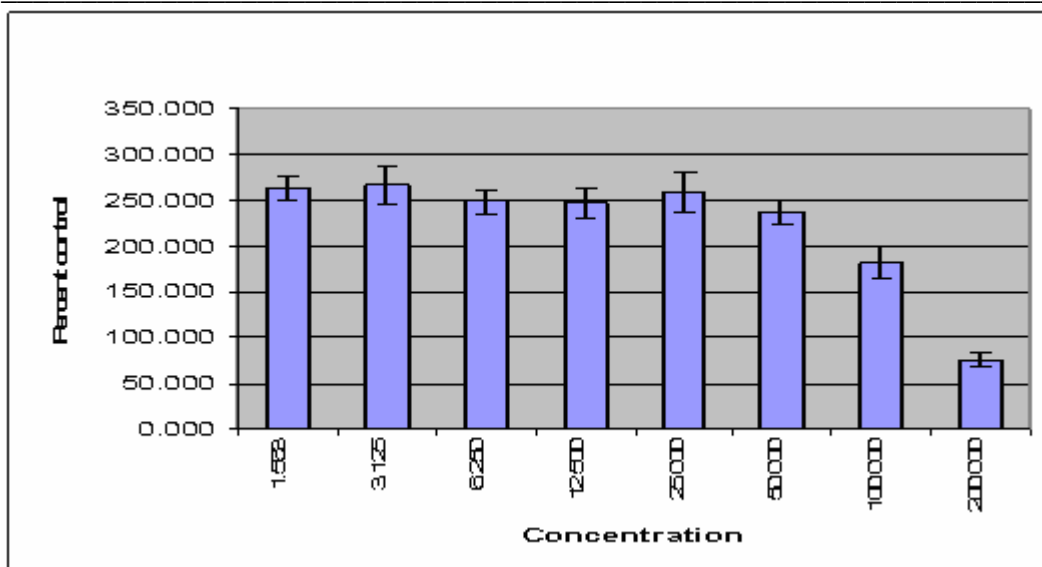
$$\text{\% Cell viability} = \frac{\text{Sample value}}{\text{Control value}} \times 100 \%$$

### 9.3 Results and discussion

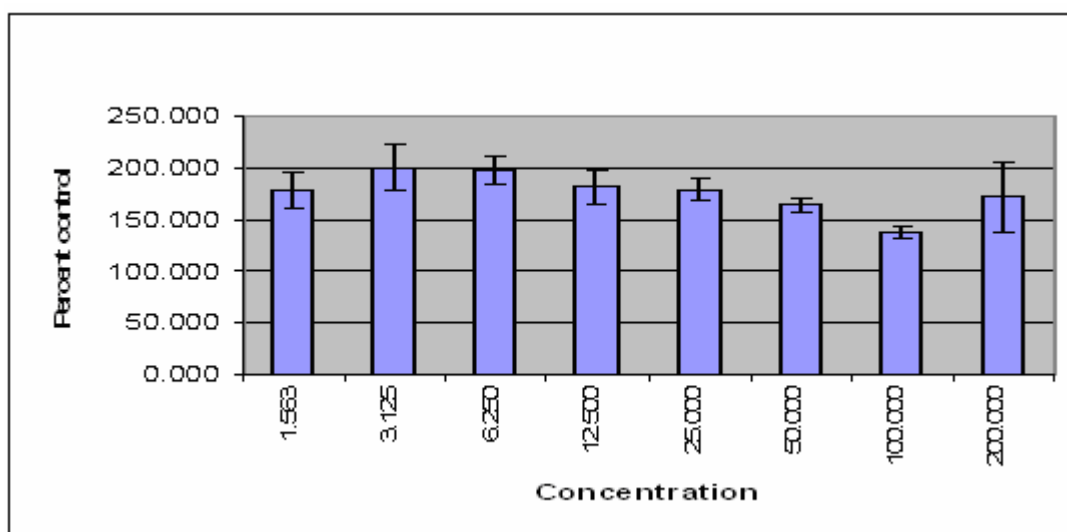
The IC<sub>50</sub> values of the acetone crude extracts of *L. javanica* and *H. opposita* and four pure compounds isolated from those species are shown in graphs (Fig 9.4 - 9.9).



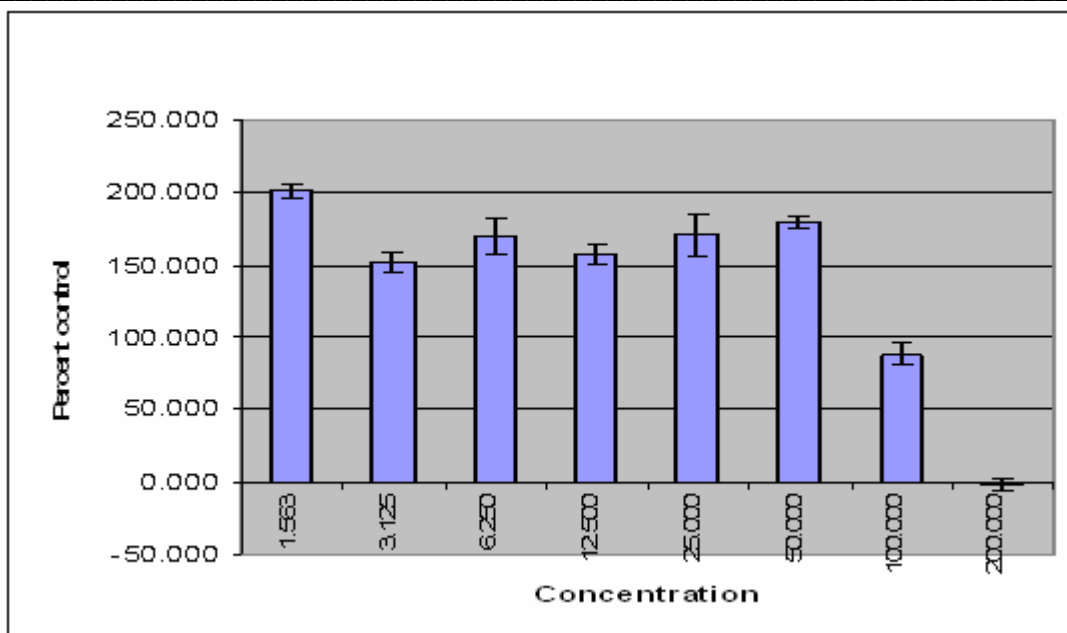
**Figure 9.2** Cytotoxicity effect of acetone extract of *Lippia javanica* on Vero cell viability. IC<sub>50</sub> values ( $\mu\text{g/ml} \pm \text{SD}$ )<sup>a</sup> of  $29.41 \pm 7.845$ . <sup>a</sup> ( $\mu\text{g/ml} \pm \text{SD}$ )=values are means  $\pm$  standard deviation.



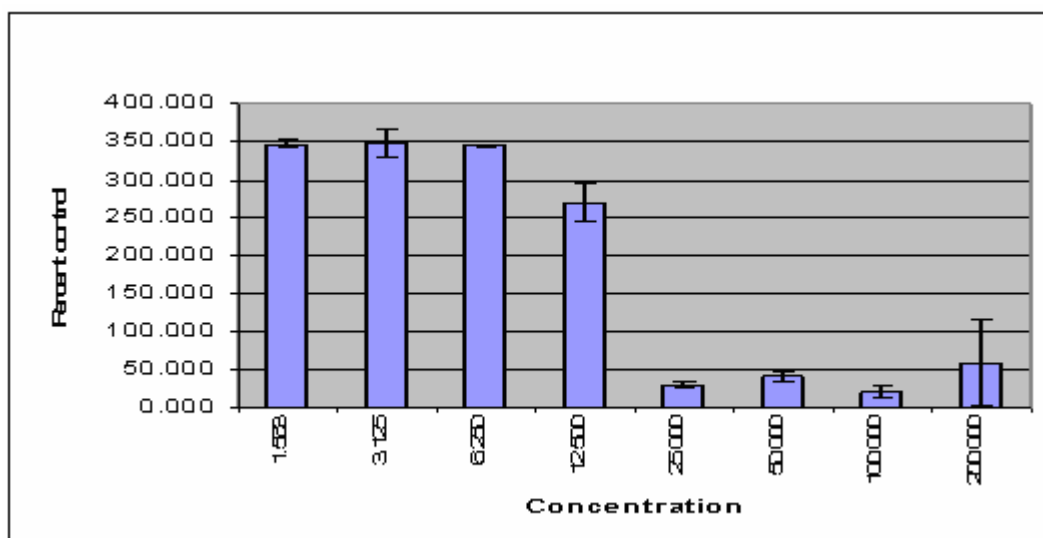
**Figure 9.3** Cytotoxicity effect of acetone crude extract of *Hoslundia opposita* on Vero cell viability.  $IC_{50}$  values ( $\mu\text{g/ml} \pm \text{SD}$ )<sup>a</sup> of  $116.8 \pm 6.162$ .



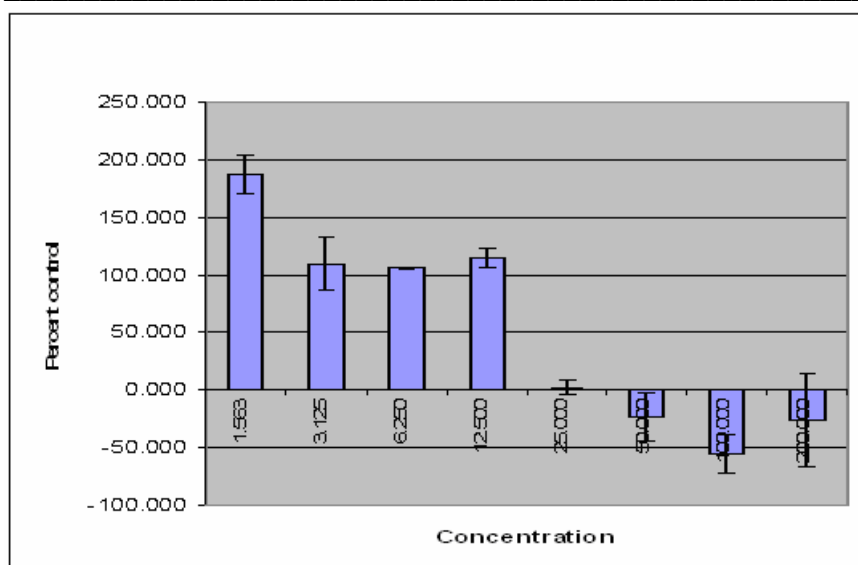
**Figure 9.4** Cytotoxicity effect of compound piperitenone on Vero cell viability.  $IC_{50}$  values ( $\mu\text{g/ml} \pm \text{SD}$ )<sup>a</sup> >200.



**Figure 9.5** Cytotoxicity effect of compound 1-(3,3-dimethoxyiranyl)-3-methyl- (2E) on Vero cell viability.  $IC_{50}$  values ( $\mu\text{g/ml} \pm \text{SD}$ )<sup>a</sup> of  $13.96 \pm 5.144$ .



**Figure 9.6** Cytotoxicity effect of compound Jacarandic acid or Euscaphic acid on Vero cell viability.  $IC_{50}$  values ( $\mu\text{g/ml} \pm \text{SD}$ )<sup>a</sup> of  $19.21 \pm 4.520$ .



**Figure 9.7** Cytotoxicity effect of compound 5,7-dimethoxy-6-methylflavone on Vero cell viability. IC<sub>50</sub> values (µg/ml ± SD)<sup>a</sup> of 2.735 ± 1.497.

The crude extracts (*L. javanica* and *H. opposita*) and isolated compounds were evaluated *in vitro* for their inhibitory ability against the growth of Vero cell line. These cell line was inhibited by all the compounds at the highest concentration tested (200 µg/ml), except the compound piperitenone. The results obtained from the calculation made from spectrophotometer readings, indicated that the crude extracts (*L. javanica* and *H. opposita*) and piperitenone compound have little or no toxicity on Vero cells by exhibiting IC<sub>50</sub> values of greater than 100 µg/ml. The compounds 5,7-dimethoxy-6-methylflavone and Jacarandic acid or Euscaphic acid showed very high toxicity by exhibiting IC<sub>50</sub> values ranging from 2.735 µg/ml to 19.21 µg/ml. This findings is consistent with observation by Ogura *et al.*(1977) which showed important *in vivo* and *in vitro* anticancer activity against P-388 lymphocytic leukaemia cells. Xu *et al.*(2003) also observed neurotoxicity of Jacarandic acid in male albino Swiss-Webster mice.

## 9.4 Conclusion

The results reported here not only provide an insight into the toxic nature of the extracts used in traditionally for the ailments treatment, but also provided an opportunity for selection of bioactive extracts for initial fractionation and further studies in antimicrobial assay. The compound “5,7-dimethoxy-6-methylflavone exhibited fifty percent inhibitory concentration (IC<sub>50</sub>) of 2.73 µg/ml. The IC<sub>50</sub> values of crude extracts of *Hoslundia opposita* and *Lippia javanica* were found to be 116.8 ± 6.16 µg/ml and 29.41 ± 7.845 µg/ml respectively. The other isolated compounds exhibited the following IC<sub>50</sub> values: piperitenone IC<sub>50</sub> >200, 1-(3, 3-dimethoxiranyl)-3-methyl- (2*E*), 13.96 ± 5.144, jacarandic acid or euscaphic acid IC<sub>50</sub> 19.21 ± 4.520 µg/ml. Among isolated compounds, Piperitenone, and among extracts, extracts of *Hoslundia opposita* seemed to show least toxicity.

Futher studies, including in vivo experiments and toxicity tests are necessary to gain a full understanding of the effectiness and possible toxic nature of these remedies

## 9.5 References

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## 10.1 Motivation for this study

For centuries, medicinal plants have been used worldwide for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate. The value of ethno-medicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine. The search for new, potentially medicinal plants is more successful if the plant is chosen on an ethnomedical basis. Many drugs have been purified from medicinal plants including antibacterial, antimycobacterial and antiviral compounds. In this study antimicrobial activity of 25 plants used in traditional medicine have been reported.

Traditional healers in the areas of Maputo, Gaza, Manica and Zambezia were consulted directly in collecting the basic ethnobotanical information about the plants studied. Based on this information 25 plants species, belonging to 20 genera and 13 families were chosen and collected in the field. Different parts (roots, stems, bark and leaves) of the selected plant species were extracted with acetone, which were subjected to assays aimed at assessing their antibacterial and antimycobacterial activities. The extracts of the plants were also assayed for their ability to inhibit the enzymes HIV-1 Reverse transcriptase (RT) and glycohydrolase ( $\alpha$ - glucosidase and  $\beta$ - glucuronidase). Searching for novel inhibitors of the HIV replication cycle is one of the main interests of numerous investigators and enormous efforts have been dedicated to find promising lead compounds. HIV-1 Reverse transcriptase (HIV-1) is one of the main targets for inhibiting the reproduction of HIV. This enzyme is



responsible for transcription of viral RNA into DNA, which is later integrated into the host cell and carries the information for the synthesis of new viral particles.

Finally, the isolation and identification of active principles was attempted using two plants species (*Lippia javanica* and *Hoslundia opposita*) which showed promising activity in the initial for antimicrobial activity.

## 10.2 Screening of plant species for biological activity

The antibacterial results presented in Chapter 2 indicate that Gram-positive bacteria were found to be more susceptible than Gram-negative bacteria to plant extracts. The weak activity shown by the extracts against Gram-negative bacteria could be due to the differences in the bacterial cell wall structures. Gram-negative bacteria are surrounded by a lipopolysaccharide layer, which provides them with additional protection against antibacterial substances. However, among the 22 plant species tested, two (*Adenia gummifera* and *Momordica balsamina*) were found to have activity against Gram-negative bacteria with a minimum inhibition concentration of 5.0 mg/ml and one (*Rhoicissus revoilli*) inhibited *E. cloacae* at 2.5 mg/ml. The antimycobacterial activity of ten plant species was investigated employing the radiometric respiratory technique BACTEC system. Bacterial cultures were grown from specimens received from the Medical Research Council (MRC) in Pretoria. A susceptible strain of *M. tuberculosis*, H37Rv reference was obtained from an American type culture collection. Four of the ten plant species showed inhibitory activity against sensitive strain of *M. tuberculosis* at a concentration of 0.5 mg/ml, which was the lowest concentration tested, (Table 2.3). Three plant extracts showed

activity against the strain at concentrations of 1.0 mg/ml and another three at 2.5 mg/ml.

The result of the anti-HIV-1 investigation of the crude extracts showed that of the seventeen plant species tested against glycohydrolase enzymes, nine extracts inhibited  $\alpha$ -glucosidase and eight  $\beta$ -glucuronidase. The inhibitory effect of ten plant extracts towards the enzyme Reverse transcriptase (RT) was shown and only two plants (*Melia azedarach* and *Rhoicissus tomentosa*) appeared to be active.

### 10.3 Isolation and identification of active compounds in plants

Out of 25 plants tested for bioassay activity it was found that *Lippia javanica* and *Hoslundia opposita* possess high antibacterial and antimycobacterial activity. In Chapters 4 and 5 the isolation and identification of bioactive compounds from *Lippia javanica* and *Hoslundia opposita* is described. Nine compounds were isolated from *L. javanica* and 3 compounds from *H. opposita*. A bioassay was applied to detect if any of the compounds inhibited the bacteria, *Mycobacterium tuberculosis* and human immunodeficiency virus (HIV) in chapters 6, 7 and 8 respectively. The antibacterial test of the isolated compounds was found to be negative at the tested concentration of 200  $\mu$ l/ml using the micro dilution method. An alkane compound was identified to be the antibacterial component, when tested using a bioautography method. The antimycobacterial activity of the isolated compounds, in Chapter 7. The MIC of 6-Methoxyluteolin 4'-methyl ether isolated from *L. javanica*, was found to be 200  $\mu$ g/ml, while the MIC of jacarandic acid, isolated from *H. opposita* was found to be 50  $\mu$ g/ml for a strain of *Mycobacterium tuberculosis*.

Three compounds were identified to be anti-HIV components (one from *H. opposita*, compound 5,7-dimethoxy-6-methylflavone, two compounds isolated from *L. javanica*, 1-(3,3-dimethyloxiranyl)-3-methyl-penta-2,4-dien-1-one and piperitenone) with the results presented in Chapter 8.

#### 10.4 Cytotoxicity of plant extracts

In order to test the safety of four bioactive compounds and both plant extracts the XTT assays were used. The results showed that the two plants species and the four compounds tested were well tolerated by Vero cells line.

#### 10.5 Conclusion

The results presented in this thesis represent an extensive investigation into plants used by Mozambican traditional healers to treat bacterial, mycobacterial and viral diseases. The value of this research lies in the scientific verification of the use of many of these plants. Two plant species and some of the compounds responsible for activity have been identified. There is much potential for future research activities in this field, as investigation of the active principles of other plants with good biological activity may yield exciting discoveries. The active compounds against HIV and *Mycobacterium tuberculosis* should be explored further for their use in disease control.