# Chapter 4

## Isolation, characterisation and bioactivities of *Plectranthus ecklonii* constituents

# Chapter 4

# Isolation, characterisation and bioactivities of *Plectranthus ecklonii* constituents

## Abstract

*Plectranthus ecklonii* Benth. is traditionally used in South Africa for treating stomach-aches, nausea, vomiting and meningitis. Bioassay-guided fractionation of *P.ecklonii* ethyl acetate extract led to the isolation of two known compounds, parvifloron D and parvifloron F. Both the isolated compounds, parvifloron D and F have not previously been reported as constituents of *P. ecklonii.* Parvifloron D and F exhibited minimum inhibitory concentration (MIC) of 15.6 and 31.25 µg/ml respectively against *L. monocytogenes.* The MICs of parvifloron D and F against a drug - sensitive strain of *Mycobacterium tuberculosis* were found to be 190 and 95 µg/ml respectively. The ethyl acetate extracts of *P. ecklonii* and its isolated compounds were tested for their activity on tyrosinase inhibition. The concentration of plant extract at which half the tyrosinase activity was inhibited ($IC_{50}$) was found to be 61.73 ± 2.69 µg/ml. The antibacterial activity of the extract of *P. ecklonii* and its isolated compounds correlates with the traditional use of the plant for various ailments such as stomach-aches, diarrhoea and skin diseases. The fifty percent inhibitory concentration of 'parvifloron D and 'parvifloron F' against vero cell lines were found to be 2.94 µg/ml and 1.56 µg/ml respectively. This is the first report on the bioactivities of extracts of *P. ecklonii* and its two constituents.

# 4.1 Introduction

Plant species belonging to the genus *Plectranthus* are found in Asia, Australia and Africa (Narukawa *et al*., 2001; Lukhoba *et al*., 2006). Several species of *Plectranthus* such as *P. barbatus, P. grandidentatus*, *P. hereroenes* have been reported to have antibacterial activity against *Staphylococcus aureus.* Diterpenes isolated from *P. grandidentus* and *P. hereroenes* were found to be active against methicillin resistant *S. aureus* (MRSA) (Matu & van Staden 2003; Gaspar-Marques *et al*., 2006). The leaves of *P. amboinicus* have been found to have antituberculosis activity (Narukawa *et al*., 2001)

*Plectranthus ecklonii* Benth. is traditionally used in South Africa for treating stomach-aches, nausea, vomiting and meningitis (Lukhoba *et al*., 2006) the symptoms associated with listeriosis infection. Leaves of the plants are also used for respiratory problems, chest complaints and coughs (TB- related problems) (Lukhoba *et al*., 2006). Aerial parts of the plant are used by people in Zimbabwe for skin diseases and skin hyper-pigmentation problems. *P. ecklonii* is widely distributed in South Africa, Australia, New Zealand, Mexico and the United States. *P. ecklonii* is a shrub which grows to about 2 metres tall. The plant grows best in semi-shade areas. The objectives of the present study were to scientifically validate the traditional uses of the plant and isolate the bioactive compounds.

# 4.2 Experimental: Materials and Methods

### 4.2.1 General experimental Procedures

$^{1}$H NMR and $^{13}$C NMR spectra were recorded using a Brucker ARX 300 or a Brucker

Avance DRX 500 MHZ using $CDCl_3$, and DMSO-$d_6$ as solvents which led to the identification of the purified compounds.

### 4.2.2 Chemicals and Reagents

XTT (2,3- bis(2-methoxy-4-nitro-5-sulfophenyl)-5[(phenylamino) carbonyl]-2-H-tetrazolium hydroxide powder, DCFDA (2,7 dichloroflourescin diacetate), 2,4,6-triprydyl-s-triazine (TPTZ) and all chemicals reagents obtained from Sigma (UK). $FeCl_3.6H_2O$, sodium sulphate, $FeSO_4$, 2-thiobarbituric acid (TBA) were obtained from Merck (Germany). *L*-Tyrosine, *L*-DOPA, tyrosinase, arbutin and Kojic acid were obtained from Sigma-Aldrich (Kempton Park, South Africa). All chemicals and solvents were of the highest commercial grade.

### 4.2.3 Plant material

Leaves of *P. ecklonii* were collected from Pretoria, South Africa. Voucher specimens of *P. ecklonii* (PRU 96396) were identified and deposited at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, South Africa.

### 4.2.4 Isolation of the bioactive compounds from *P. ecklonii*

The leaves of *P. ecklonii* (550 g) were extracted with ethyl acetate (2 x 2L) for 48 hours, at room temperature (± 25°C). The ethyl acetate extract was filtered and concentrated to dryness using Rotavapor. The total extract (110 g) was subjected to silica gel column chromatography (CC, size 7 cm x 120 cm) using hexane/ethyl acetate mixtures of increasing polarity (0 to 100%)

(Figure 4.1). Similar fractions were combined into 5 main fractions based on the TLC profile (Figure 4.2). The five fractions were tested against *L. monocytogenes* and it was found that 2 fractions, fraction IV and V exhibited good antilisterial activity. Fraction V (2.9 g) was subjected to a Sephadex LH-20 column using dichloromethane/methanol mixtures (100 % dichloromethane, 99 %, 2 %, 3 %) (v/v) as an eluent to give compound P **1** (yield 2.31g, 0.42 %). Fraction IV (2.5 g), under the same conditions gave pure compound P **2** (yield 937 mg, 0.17 %).

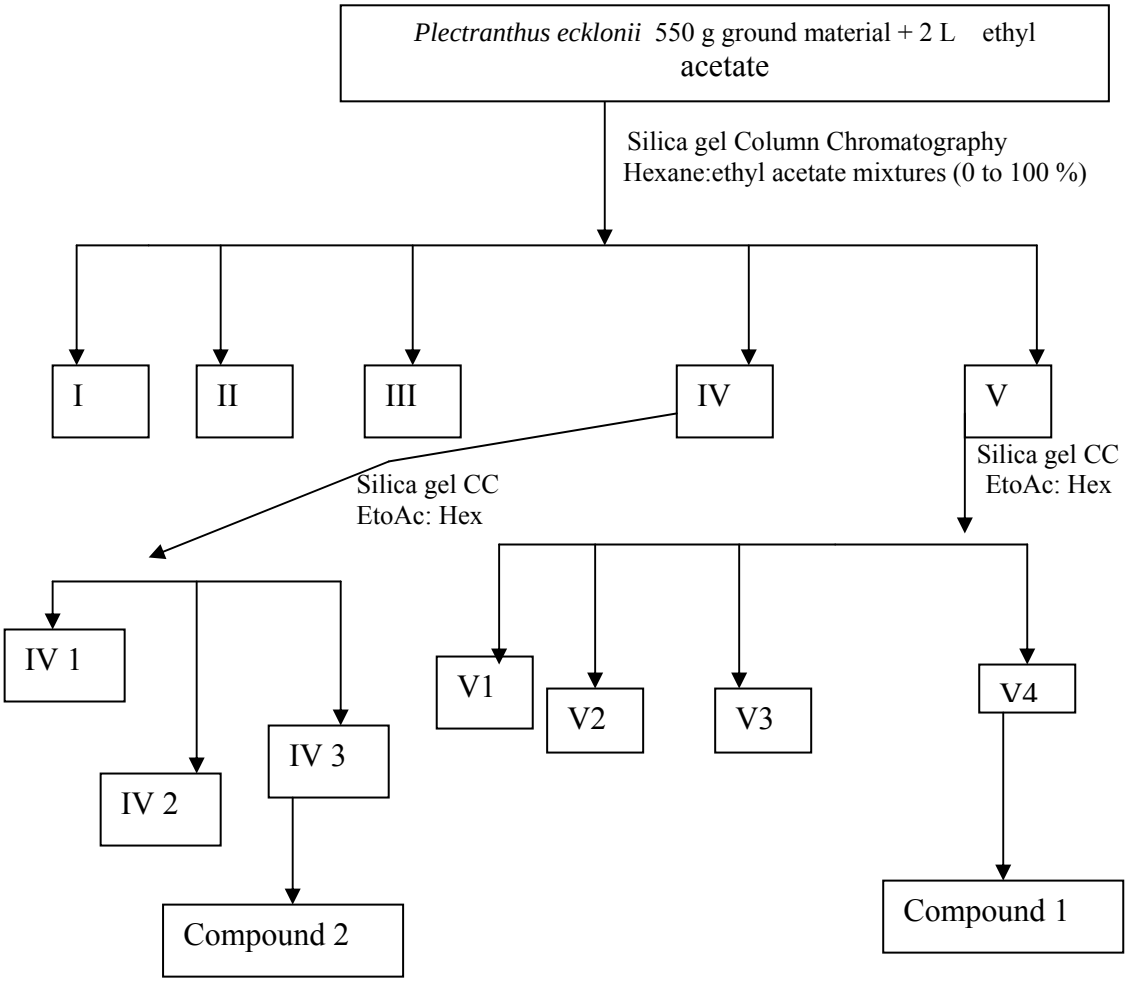
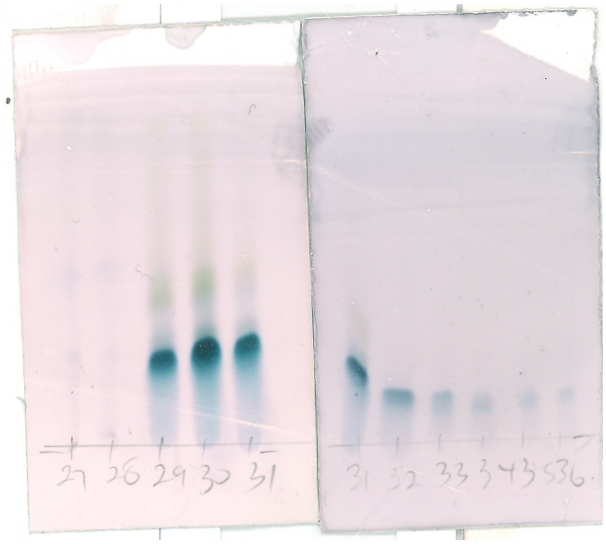



**Figure 4.1** Schematic representation of the isolation process of the bioactive compounds from *Plectranthus ecklonii*

**(a)**




**(b)**

**(c)**

**Figure 4.2** (a) Silica gel column chromatography of the ethyl acetate extracts of *P. ecklonii*, (b) fractions obtained by column chromatographic purification, spotted onto the TLC plates and (c) direct bioassay on TLC plates.

### 4.2.5 Activity of samples against *Listeria monocytogenes* and other bacteria

The bacterial culture of *L. monocytogenes* (LMG 21263) was activated by transferring a loop full from brain heart infusion (BHI) slants into tryptone soya broth (TSB) followed by incubation at 37 °C for 24 hours (Alzoreky & Nakahara, 2003). The optical density of the culture was adjusted to 0.1 at 590 nm using fresh broth to give a standard inoculum of $10^6$ colony forming units (CFU/ml). The bacterial count was confirmed by plating out on TSA and Mueller–Hinton agar plates incubated at 37 °C for 24 hours. Stock cultures were maintained at -70 °C (Alzoreky & Nakahara, 2003). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) determination were done as stated previously (Eloff, 1998; Mathabe *et al*., 2008). Briefly, the crude extracts and purified compounds were dissolved in 2.5% DMSO to obtain a stock solution. The DMSO (2.5%) was used as control to test the inhibition of the bacteria. The concentrations of the crude extract and the compounds tested ranged from 7.81 to 1000 µg/ml. Double fold serial dilutions of each sample was performed. Tests were done in triplicate. The plates were sealed and incubated at 37 ºC for 24 hours. The MIC of the samples was determined by adding 40 µl of (0.2 mg/ml) *p*-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, South Africa) to microtitre wells and the re-incubated at 37 ºC for 1 hour (Eloff, 1998; Mativandlela *et a*l., 2006) to indicate the presence of bacterial growth (pink colour) or inhibition of bacterial growth (no colour). The MIC was defined as the lowest concentration of the extract that caused no colour change and showed complete inhibition of bacterial growth. The minimum bactericidal concentration (MBC) was determined by transferring 50 µl of the sample from the wells which did not show bacterial growth during the MIC assays (without INT), to 150 µl of the freshly prepared broth. The plates were re-incubated at 37 ºC for 48 hours. The MBC was regarded as the lowest concentration which did not give rise to a colour change

after INT was added as described above in the MIC assay. Erythromycin was used as a drug control for *L. monocytogenes.*

Culture of *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Pseudomonas aeruginosa* (ATTCC 27853) were each transferred on to nutrient agar slant and the culture was then recovered for testing by growing on a nutrient broth (BIOLAB, South Africa) for 24 hours at 37 ºC . Ciprofloxacin was used as a drug control. The MIC and MBC was determined as stated above.

### 4.2.6 Antimycobacterial bioassay

*Mycobacterium smegmatis* ($MC^2$ 155) and a drug susceptible strain of *M. tuberculosis* H37Rv (ATCC 27264) were acquired from *American* Type, MD, USA Culture Collection. The microplate dilution method was used for the testing of the samples against *M. smegmatis* in 96-Well microtitre plates according to Salie *et al*. (1996). The susceptibility testing of *M. tuberculosis* was done with the radiometric respiratory technique using the BACTEC 460 system (Becton Dickinson Diagnostic Instrument, Sparks, MD) as described previously (Lall & Meyer,1999; Lall & Meyer, 2000; Lall & Meyer, 2001). The crude extracts and purified compounds were dissolved in 2.5% DMSO in sterile 7H9 broth to obtain a stock concentration of 1.250 mg/ml. The DMSO (2.5%) was used as control to test the inhibition of bacteria. The final concentration ranged from 9.76-312.50 µg/ml.

### 4.2.7 Antityrosinase assay

Melanin is a key pigment responsible for skin and / or hair colour. Its production is

catalysed by an enzyme called 'tyrosinase'. Since, traditionally the paste made of the leaves of *P. ecklonii* are used by South Africans for skin-hyperpigmentation problem, it was therefore, decided to test the extract and purified compounds of *P. ecklonii* for their activity on tyrosinase inhibition. The method as previously described (Nerya *et al*., 2003) was followed with modifications. The drug control used was the kojic acid (Kim *et al*., 2004). Briefly each sample (crude extract or compound) was dissolved in DMSO to a final concentration of 20 mg/ml. The final percentage of DMSO was 1.5% for both crude extract and pure compound. The sample stock solution was then diluted to 200 µg/ml in a 50 mM potassium sulphate buffer. Seventy microlitre of each sample dilution was combined with 30 µl of tyrosinase (333 units/ml in phosphate buffer) (This was done in triplicate) in a 96-well microtitre plates. After the elapse of five minutes 110 µl of the substrate (2 mM L-tyrosinase or L-DOPA) was added to each well. The final concentrations of the samples ranged from 200 to 12.5 µg/ml. The plates were then incubated at room temperature for 30 min. Optical densities of the reaction mixtures in the wells were then recorded at 492 nm with the BIO-TEK power Wave XS multi well plate reader.

### 4.2.8 Cytotoxicity test

The method as stated in section 3.2.4 of this thesis was followed to determine the the cytotoxicity test.

### 4.2.9 Effect of the crude extract of *P. ecklonii* and its compounds on listerial biofilms

The method as stated in section 3.2.4 of this thesis was followed to determine the listerial biofilm formation on exposure to the purified compounds.

## 4.3 Results and Discussion

The two known compounds were isolated from ethyl acetate extract of *P. ecklonii*. The respective NMR spectra are as follows:

Compound P **1**

The compound P **1** was isolated as orange crystals from the non polar part of the extract of *P. ecklonii*. The structure of compound P **1** was established to be parvifloron D based on the spectroscopic data (Figure 4.1). $^{1}$H NMR signals at 6.40 (d, J = 6.8; H - 6); 6.75 (d, J = 6.8, H – 7); 6.94 (d, J =0.5, H-14); 5.59 (s, H-2β), five methyl groups signals at 1.15, 1.19 (each d, J =6.4, Me-16,17) 1.29 (s, Me-18), 1.41 (s, Me-19), 1.69 (s, Me–20). The $^{13}$C- NMR indicated the presence of 27 carbons, 20 of which of the diterpene skeleton and the rest represent the signals of *p*-benzoic acid derivatives (Figure 4.3). The signal of 2β was shifted to low field due to the esterification of the *p*-benzoic acid with the OH at the same position. This was confirmed by the comparison of the obtained data with those of published in literature. Compound P**1** is a terpene. Compound P **1** (parvifloron D) was previously isolated from *P. strigosus* (Gaspar-Marques *et al*., 2008). Compound P **1** has been reported to have antibacterial activity against *Staphylococcus* and *Enterococcus* species, including methicillin- and vancomycin-resistant strains (Simões *et al*., in press). Compound P **1** has not been tested on *L. monocytogenes*.

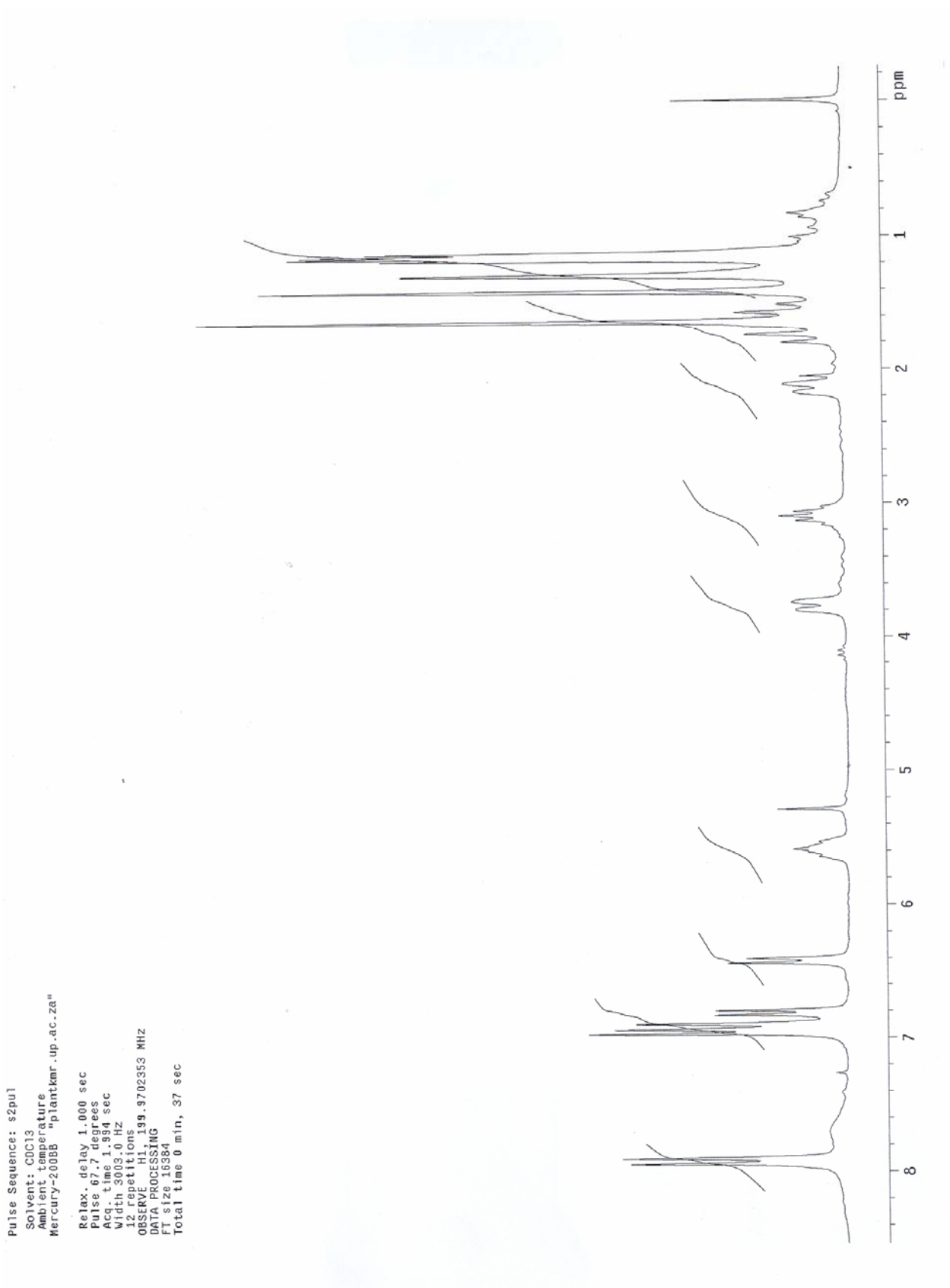



**Figure 4.3** $^{1}$HNMR sectrum of compound P **1** (parvifloron D) isolated from *P. ecklonii*

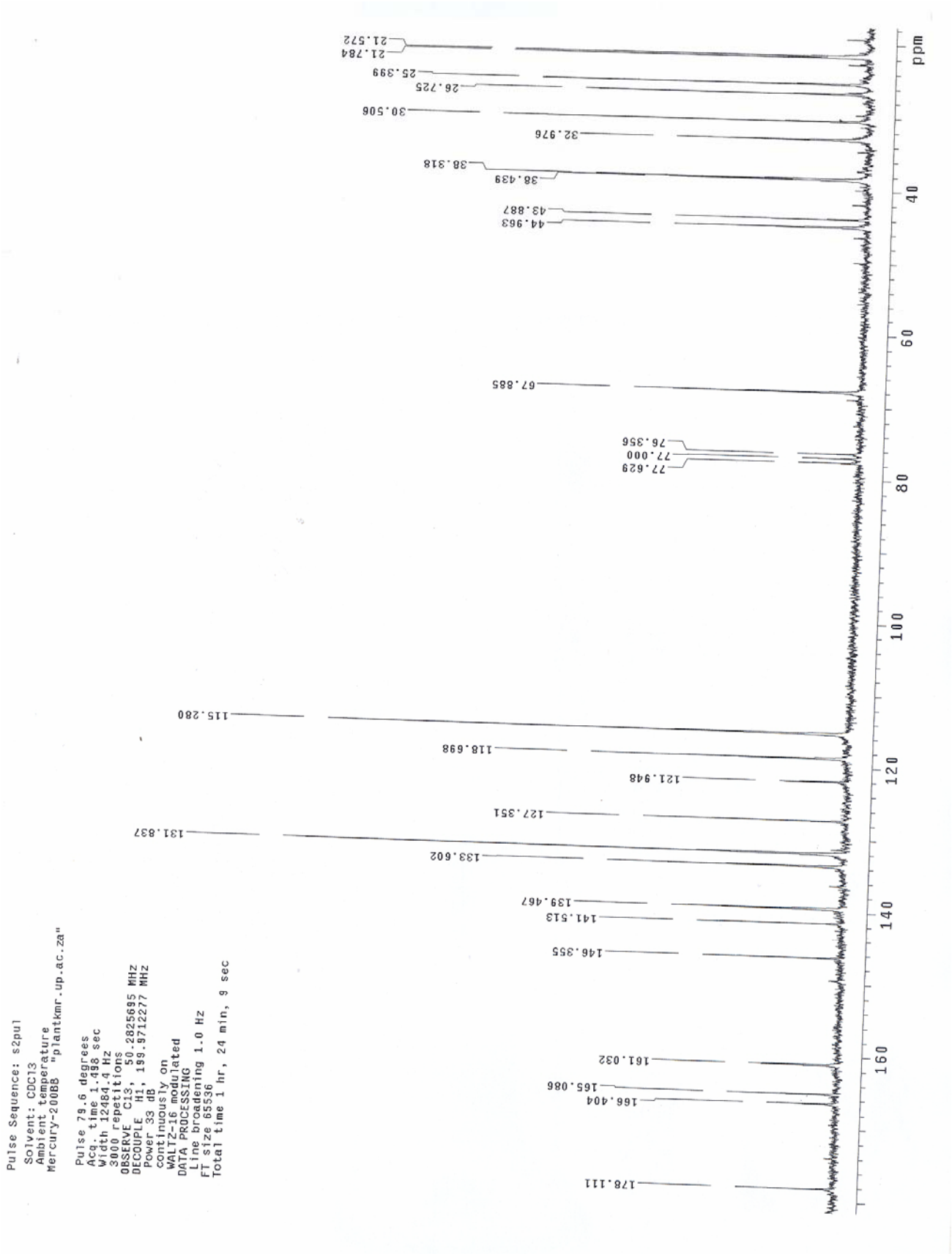



**Figure 4.4** $^{13}$CNMR spectrum of compound P **1** (parvifloron D) isolated from *P. ecklonii*

Compound P **2**

Compound P **2** (parvifloron F) showed NMR data (Figures 4.3 and 4.4) similar to those of compound P **1** (parvifloron D) except for the side chain, which showed signals of 1,3,4-trisubstituted benzoic acid patterns instead of 1,4-disubstituted benzoic acid. The data obtained also were compared with those, which published in literature. Compound P **2** belongs to the diterpenes group of compounds. Compound P **2** (parvifloron F) was previously isolated from *P. nummularius* (Narukawa *et al*., 2001). Not much has been reported in literature about the antibacterial activity of compound P **2** except its anti-oxidative activity (Narukawa *et al*., 2001). Compound P **2** has not been tested on *L. monocytogenes*. Both the isolated compounds, Parvifloron D and F have not previously been reported as constituents of *P. ecklonii*. Figure 4.5 shows the chemical structures of compounds P **1** and P **2.**

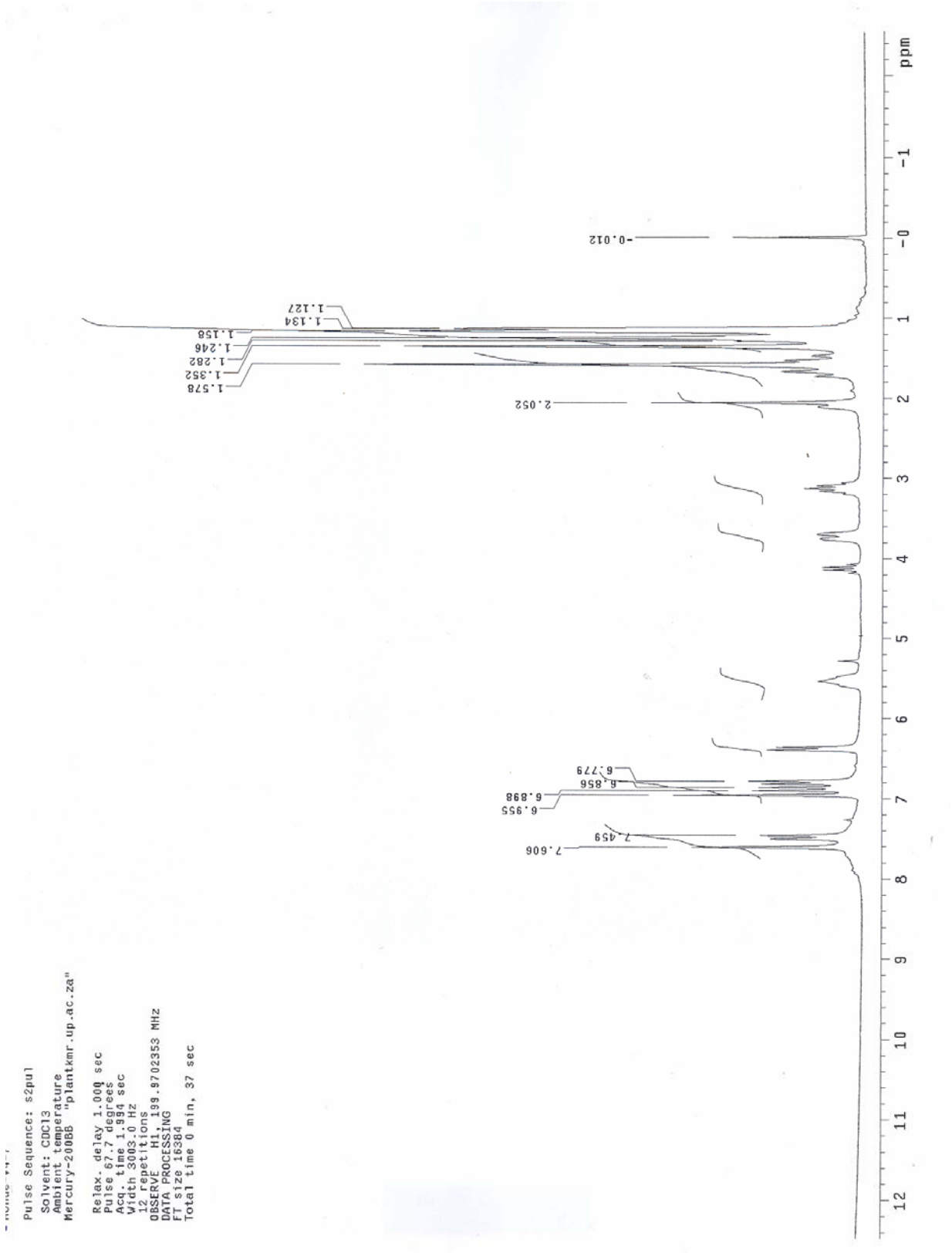



**Figure 4.5** [1]HNMR spectrum of compound P **2** ( parvifloron F) isolated from *P. ecklonii*

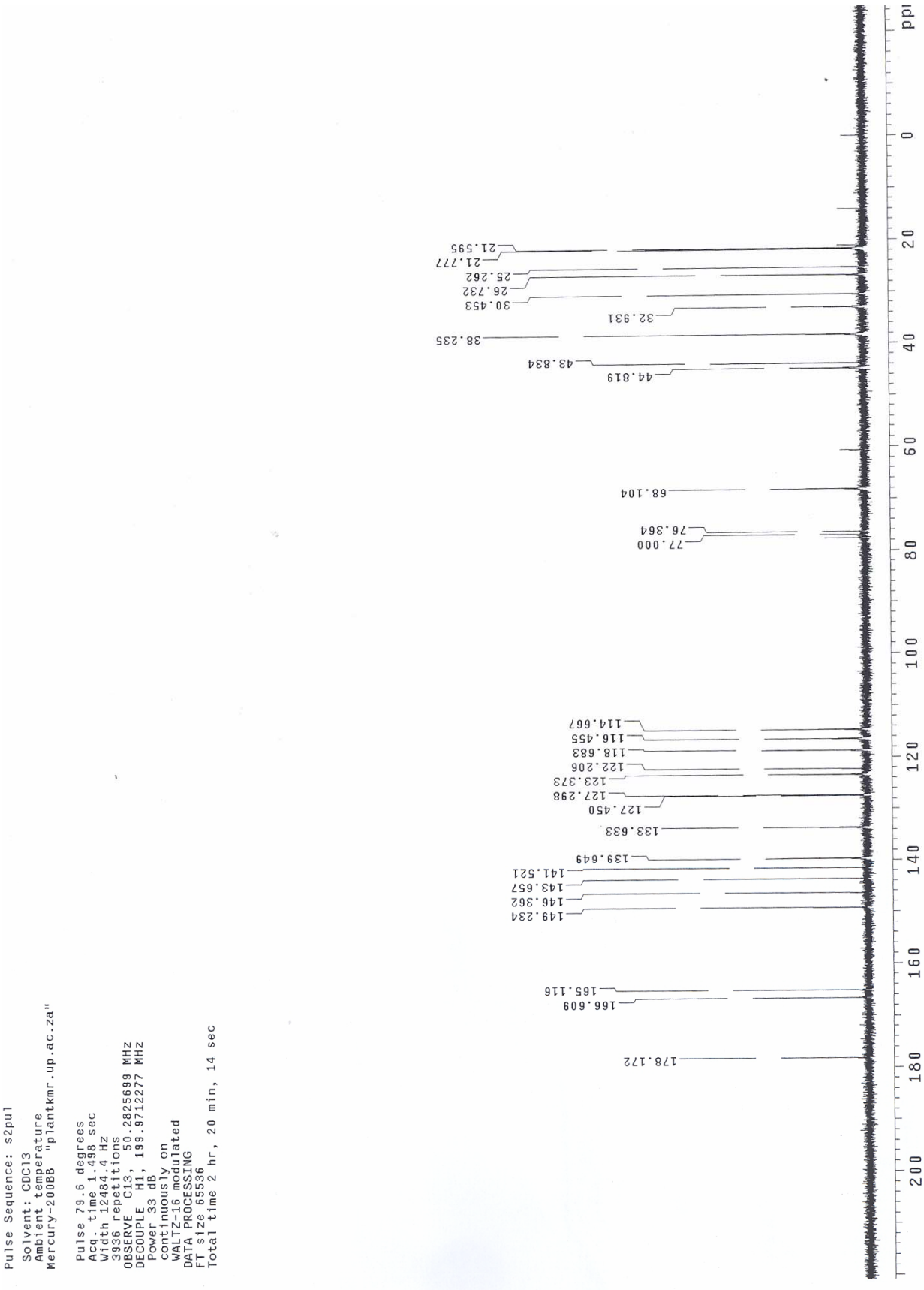
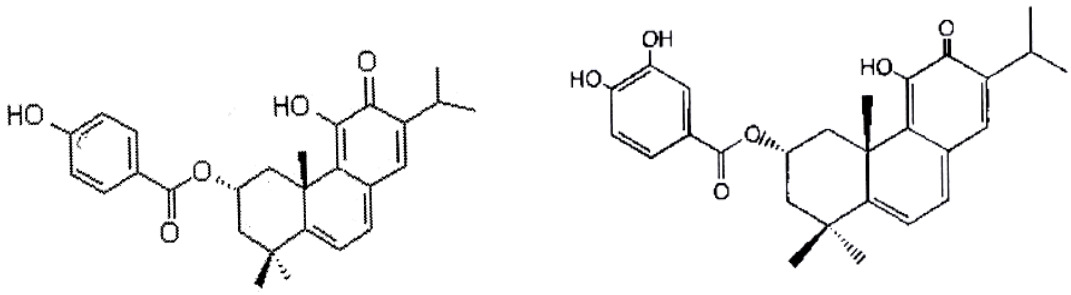



**Figure 4.6** $^{13}$C NMR spectrum of compound P **2** (parvifloron F) isolated from *P. ecklonii*




P **1** P **2**

**Figure 4.7** Chemical structures of P (**1**) parvifloron D and P (**2**) parvifloron F

The ethyl acetate extracts of the leaves of *P. ecklonii* showed good activity against *Listeria monocytogenes* (LMG 21263) exhibiting a minimum inhibitory concentration (MIC) of 500 µg/ml. In previous studies the MIC of other plants have been found to be generally higher than the one observed in this study. Alzoreky and Nakahara (2003) reported the MIC values of methanolic extract of *Artemisia absithium* and eighteen other plants as ranging from 1.32 mg/ml to 2.64 mg/ml against *L. monocytogenes*.

Column chromatographic purification of the ethyl acetate extracts of the aerial parts of *P. ecklonii* resulted in five major fractions. The five fractions were tested against *L. monocytogenes* and it was found that 2 fractions, fraction IV and V exhibited a MIC of 62.5 µg/ml and 125 µg/ml respectively. Fractions I – III were not found to be active against *L. monocytogenes* at the highest concentration (500 µg/ml) tested. The two known compounds that were isolated from *P. ecklonii* were compound **1** (from fraction IV) and compound **2** (from fraction V)

Other compounds that have been previously isolated from *P. ecklonii* are 'ekclonoquinone A', 'ecklonoquinone B', 4',5-dihydroxy-6,7-dimethoxyflavanone, nepetoidin A and B (Grayer *et al*., 2003; DNP, 2010) Nepetoidin A and B have been reported to exhibit antifungal activity against *Aspergillus niger* (Grayer *et al*., 2003).

Besides the above data, not much is known about phytochemical properties of this plant. The MICs of the compounds P **1** and P **2** against *L. monocytogenes* were found to be 15.60 µg/ml and 31.25 µg/ml respectively. These results are comparable to the MICs of 125 µg/ml obtained for both (*Heracleum sphondylium* subsp. *ternatum*) and its main component, 1-octanol against *L. monocytogenes* (İscan *et al*., 2003).

The MICs of *P. ecklonii* ethyl acetate extracts against Gram-positive and Gram-negative bacteria species ranged from 125.0 to 1000.0 µg/ml (Table 4.1).

Compounds purified from *P. ecklonii* ethyl acetate extracts were found to be active at a low concentration of 31.3 µg/ml against some of the bacterial species tested. The MIC of other *Plectranthus* species, such *P. cylindraceus* oil against *E. coli* was found to be 125 µg/ml (Marwah *et al*., 2007) which is similar to the results obtained in the present study. In the previous study it has been reported that *E. globulus* was found to have antibacterial activity against as *S. aureus* and *E. coli* (Välimaa *et al*., 2007). .*P ecklonii* is traditionally used to treat skin infections in Zimbabwe (Lukhoba *et al*., 2006). The traditional use of *P. ecklonii* for skin infections could be possibly linked to the antibacterial activity of parvifloron D (P **1**) and parvifloron F (P **2**) against *S. aureus*. *Plectranthus* species have been traditionally used in folk medicine to treat digestive and stomach-ache (Lukhoba *et al*., 2006). The activity of *P. ecklonii* against *Escherichia coli* observed in the present study justifies the use of *Plectranthus* species in traditional medicine in the treatment of gastro-enteric infections.

**Table 4.1**: Antibacterial and antityrosinase activity of the ethyl acetate extracts of the aerial parts of *Plectranthus ecklonii* and its isolated compounds. Cytotoxicity of the extract and the isolated compounds against Vero monkey cells.

| Bacteria tested | **MIC / MBC [a] ( µg/ml)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ethyl acetate extract *of P. ecklonii* | Compound **1** (parvifloron D*)* | Compound **2** (parvifloron F ) | RA[b] | Kojic acid[c] | Doxorubicin[d] | Isoniazid[e] |
| *E. coli* | 125.0<br>500.0 | 31.25<br>31.25 | 31.25 62.5 | 1.2<br>1.2 | | | |
| *E. faecalis* | 250.0<br>250.0 | 31.25 62.5 | 62.5 62.5 | 18.75<br>18.75 | | | |
| *L. monocytogenes* | 500.0<br>1000.0 | 15.6<br>31.25 | 31.25<br>62.5 | 1.2<br>1.2 | | | |
| *S. aureus* | 250.0<br>250.0 | 31.25<br>31.25 | 15.6<br>31.25 | 9.4<br>9.4 | | | |
| *P. aeruginosa* | 250.0<br>250.0 | 31.25<br>31.25 | 31.25<br>62.5 | 18.75<br>18.75 | | | |
| *M. smegmatis* | 78.12<br>625.0 | 39.06<br>156.25 | 39.06 78.1 | 0.61<br>1.22 | | | |
| *M. tuberculosis* | 380.0<br>>380.0 | 190.0<br>190.0 | 95.0<br>190.0 | | | | 0.12<br>0.12 |
| $IC_{50}$ (Antityrosinase activity | 61.73 ± 2.69 | Na[g] | Na | | 2.145± 0.08 | | |
| $IC_{50}$ (cytotoxicity testing ) | < 3.1 | 2.935 | < 1.56 | | | 0.5449 | |
| SI[f] ($IC_{50}$/MIC ) | 0.06 | 0.19 | 0.05 | 8.46 | | | |

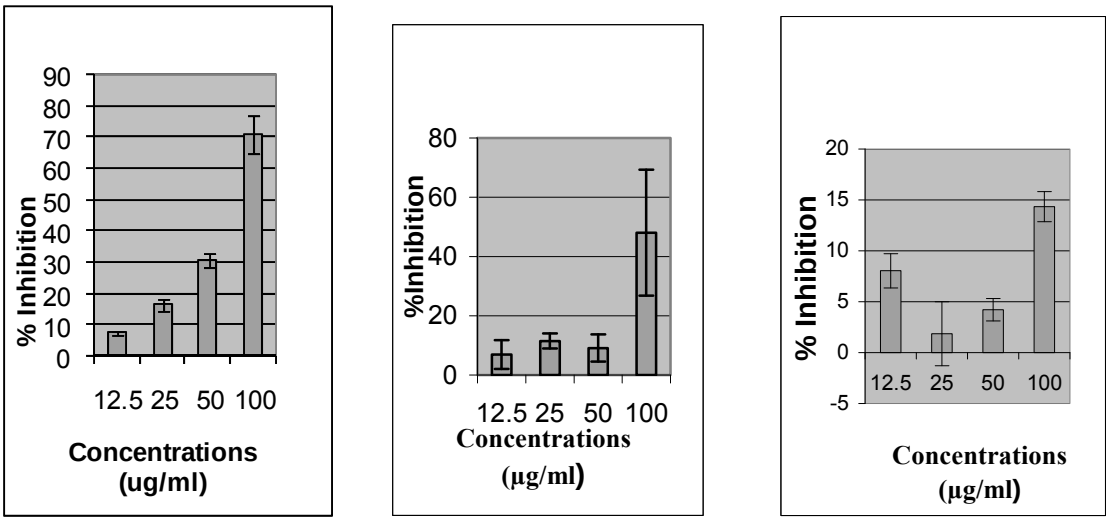
[a]MIC: Minimum inhibitory concentration; [b]RA: Reference antibiotics: erythromycin (positive drug control for *Listeria monocytogenes*), ciprofloxacin (positive drug control for *Mycobacterium smegmatis, Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas. aeruginosa*); [c]Kojic acid (positive drug control for antityrosinase bioassay); [d]Doxorubicin (positive drug control for cytotoxicity testing); [e]Isoniazid (positive drug control for *Mycobacterium. tuberculosis*); [f]SI: ( $IC_{50}$/MIC) Selectivity index;

[g]Na : not active at the high concentration (100 µg/ml) tested.

The anti-mycobacterial activity of the investigated crude extract of *P. ecklonii* and its isolated compounds are shown in Table 4.1 Both compounds P **1** and P **2** showed good activities against *M. tuberculosis*. The MIC of 95 µg/ml exhibited by compound **2** in the present study was similar to the reports on the inhibitory activity of shinanolone and mamegakinone against *Mycobacterium tuberculosis* H37RV strain (Van der Kooy *et al*., 2006). The MIC of the *P. ecklonii* crude extract against *M. tuberculosis* H37RV strain (380 µg/ml) was found to be comparable to those of previous studies on a number of *Helichrysum* species that exhibited MICs of 0.5 mg/ml (500.0 µg/ml) (Marwah *et al*., 2007). A number of other plants such as *Pelargonium reniforme*, *P. sidoides*, *Achillea millefolium*, *Ageratum corimbosum*, and *Anoda cristata* have been reported to have MIC values ≥ 1.0 mg/ml against *M. tuberculosi* (Jiminez-Arellanes *et al*., 2003; Mativandlela *et al*., 2006). The crude extract of *P. ecklonii* exhibited an MIC of 78.12 µg/ml against a non pathogenic *Mycobacterium* species i.e., *M. smegmatis*. Our results are in agreement with previous findings where it was stated that extracts of *Mentha piperita*, *Cinnamomum zeylanicum* gave an MIC of 500.0 µg/ml against *M. smegmatis* (Newton *et al*., 2002).

Both compounds P**1** and P **2** also showed good anti-mycobacterial activity against *M. smegmatis* at a concentration of 39.06 µg/ml (MIC). The selectivity index (SI = $IC_{50}$/MIC) calculated to 0.1 and 0.05 for parvifloron D (P **1**) and parvifloron F (P **2**) respectively. Parvifloron D (P **1**) had the highest SI value compared to parvifloron F (P **2**) and the *P. ecklonii* ethyl acetate crude extract in the present study. This is in accordance with the literature (Kovala-Demertzi *et al*., 2009) where the compound with the highest actvity was also found to be more selective (high SI value) than the compound with less activity.

The ethyl acetate extract of *P. ecklonii* and its isolated compounds were tested for their activity on tyrosinase inhibition (Figure 4.8). The concentration of plant extract at which half the tyrosinase activity was inhibited ($IC_{50}$) was 61.73 ± 2.69 µg/ml.




(a) (b) (c)

**Figure 4.8** Dose response curve of antityrosinase activity of (a) *P. ecklonii* crude extracts (b) parvifloron D (P **1**), (c) parvifloron F (P **2**)

During cytotoxicity evaluation (Figures 4.9 and 4.10) the fifty percent inhibitory concentration of 'parvifloron D and 'parvifloron F' against vero cell lines were found to be 2.94 µg/ml and 1.56µg/ml respectively (Table 4.1).

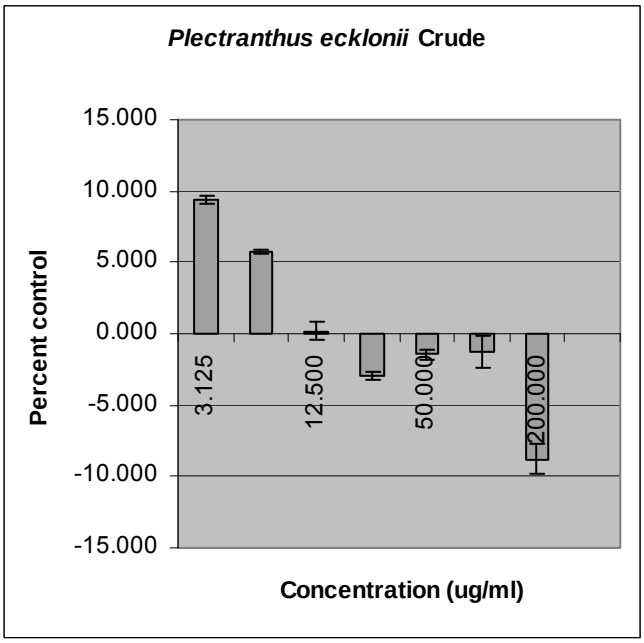
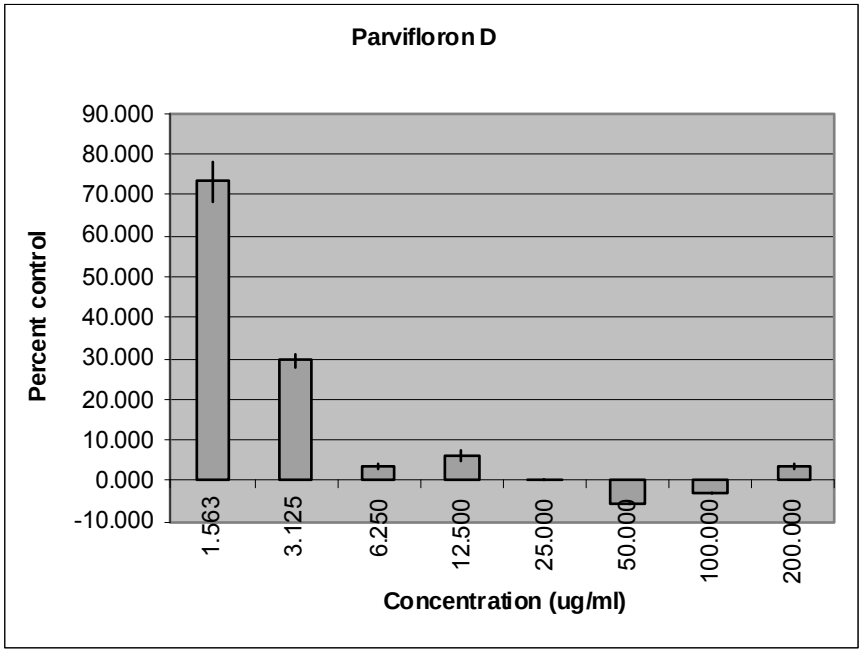



**Figure 4.9** Dose response curve of (a) *P. ecklonii* ethyl acetate extract against Vero cell lines.

Results of the cytotoxicity tests showed that isolated compounds were toxic as shown by the fifty percent inhibing concentration ($IC_{50}$ values) against monkey kidney Vero cell lines (Table 4.1). From the graphs that shows cytotoxicty, the viability cells for *P. ecklonii* crude and pure compound, parvifloron F (P **2**) the Vero cells viability never reached up to 50%. This has had an impact on the calculation of the $IC_{50}$ values .The reason could be that the the extract and the compound were too toxic to the cells. The negative values from the graph are due to the colouraton of the extracts. .It has been reported that compounds with the lowest minimal inhibitory concentrations (MIC) are also the most cytotoxic against green monkey Vero cell line (Zentz *et al*., 2004). This is in contrast with literature were compounds with lower MICs showed least cytotoxicity against the Vero cells (Mathabe *et al*., 2008). The antibacterial activity of the extract of *P. ecklonii* and its isolated compounds correlates with the traditional use of the plant for various ailments such as stomacha-ches, diarrhoea and skin diseases.




(a)

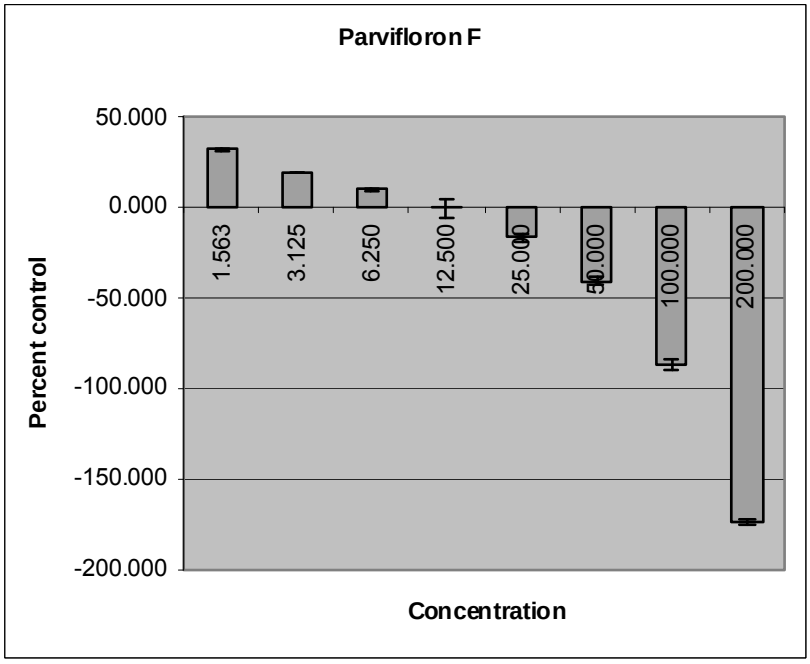
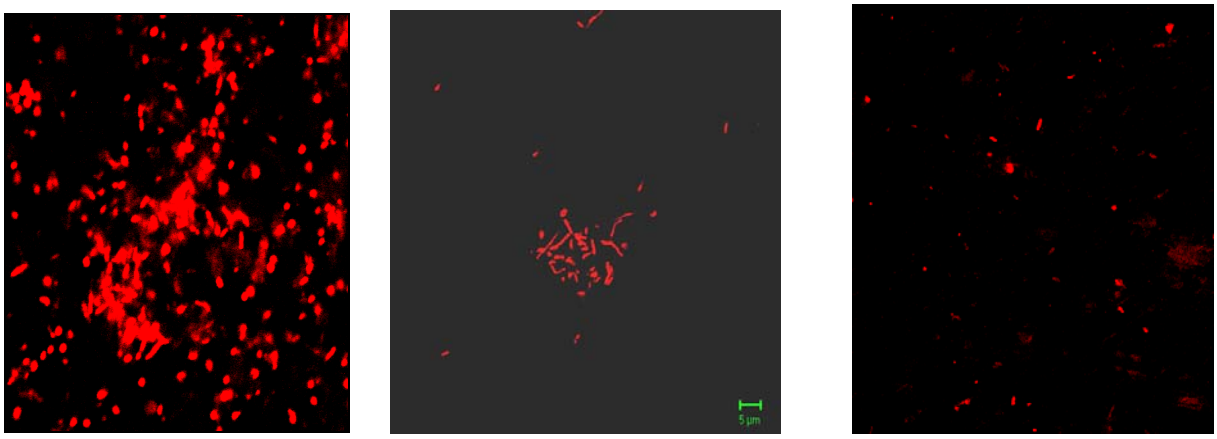



**Figure 4.10** Dose response curve of (a) parvifloron D (P **1**), (b) parvifloron F (P **2**) against Vero cell line

The untreated listerial cells formed a dense biofilm while the cells treated with parvifloron D (P **1**) disrupted the formation of a listerial biofilm (Figure 4.11 (c)). In a previous study it was reported that the use of American cranberry (*Vaccinium macrocarpon*) showed the activity against the foodborne pathogen, *L. monocytogenes* (Chi-Hua Wu, *et al*, 2008).

Figure 4.11 (b) showed a slight reduction in the development of the listerial biofilm. In this study both parvifloron D (P **1**) and parvifloron F (P **2**) had greater activity in the disruption of the *L. monocytogenes* biofilm. The aggregation of cells which were exposed to parvifloron D (P **1**) and parvifloron F (P **2**) was reduced from 25µm as observed in untreated cells to < 10 µm in diameter in treated cells.



(a) (b) (c)

**Figure 4.11** CSLM images of *L. monocytogenes* (LMG21263) biofilms without treatment (negative control) (a), (b) after treatment with *P. ecklonii* and (c) after treatment compound, 'parvifloron D' (P **1**). *L. monocytogenes* biofilm formed on coverslips were examined with Zeiss LSM 510 META confocal scanning laser microscope using a water immersion lens.

In the previous study, knotwood and bark extracts of *Picea maritma* and *Eucalyptus globulus* respectively, were found to have antibacterial activity against *L. monocytogenes*. The flavonoid, pinocembrin has been previously reported to be active against *L. monoctogenes* (Välimaa *et al*., 2007). In another study it has been previously reported that cranberry, cloudberry, raspberry and strawberry have shown activity against *L. monocytogenes* (Puupponen-Pimiä *et al*., 2005).

*P. ecklonii* has not been tested before on *L. monocytogenes*. *P. ecklonii* has been tested on *Streptococcus sobrinus*, *S. mutans*, *Staphylococcus aureus*, *S. epidermis*, *Bacillus subtilis* and *Micrococcus luteus* (Rabe and Van Staden, 1998; Figueiredo *et al*., 2010). *P. ecklonii* has been reported to be medicinally used for skin problems in South Africa (Lukhoba *et al*., 2006).

## 4.3 References

ALZOREKY, N.S., NAKAHARA, K., 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology 80, 223-230.

CHI-HUA-WU, V., QIU, X., BUSHWAY, A., HARPER, L., 2008. Antibacterial effects American cranberry (*Vaccinium macroccarpon*) concentrate on foodborne pathogens. Food Science and Technology 41, 1834- 1841.

ELOFF, J.N., 1998 A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711-713.

FIGUEIREDO, N., DE AQUIAR, S.R.M.M., FALÉ, P.L., ASCENCÃO, L., SERRALHEIRO, M.L.M., LINO, A.R.L., 2010. The inhibitory effect of *Plectranthus barbatus* and *Plectrantus ecklonii* leaves on the viability, glucosyltransferase activity and biofilm formationof *Streptococcus sobrinus* and *Streptococcus mutans*. Food Chemistry 119, 664-668.

GASPAR-MARQUES, C., RIJO, P., SIMÕES, M.F., DUARTE, M.A., RODRÍGUEZ, B., 2006. Abietanes from *Plectranthus grandidentatus* and *P. hereroensis* against methicillin - and vancomycin-resistant bacteria. Phytomedicine 13, 267-271.

GASPAR-MARQUES, C., SIMÕES, M.F., VALDEIRA, M.L., RODRÍGUEZ, B., 2008. Terpenoids and phenolics from *Plectranthus strigosus*, bioactivity screening. Natural Product Research 22, 167-177.

GRAYER, R.J., ECKERT, M.R., VEITCH, N.C., KITE, G.C., MARIN, P.D., KOKUBUN, T., SIMMONDS, M.S.J., PATON, A.J., 2003. The chemotaxonomic significance of two ] bioactive caffeic acid esters, nepetoidins A and B, in the *Lamiaceae*. Phytochemistry 64,

519-528.

İSCAN G, DEMIRCI F, KÜRKCÜOĞLU M, KIVAC M, BASER H C., 2003. The Bioactive Essential Oil of *Herecleum sphondylium* L. subsp. Ternatum (Velen.) Brummitt. Presented at 33rd Symposium on Essential Oils (33rd ISEO) on 4-7 September, 2002 in Lisbon, Portugal.

JIMINEZ-ARELLANES, A., MECKESI, M., RAMIREZ, R., TORRES, J., LUNA-HERRERA, J., 2003. Activity against multidrug resistant *Mycobacterium tuberculosis* in Mexican plants. Phytotherapy Research 17, 903-908.

KIM, H., CHOI, J., CHO, J.K., KIM, S.Y., LEE Y., 2004 Solid-phase synthesis of kojic acid-tripeptides and their tyrosinase inhibitory activity, storage stability, and toxicity. Bioorganic & Medicinal Chemistry Letters 14, 2843-2846.

KOVALA-DEMERTZI, D., DOKOROU, V., PRIMIKIRI, A., VARGAS, R., SILVESTRU, C., RUSSO, U., DEMERTZIS, M.A., 2009. Organotin meclofenamic complexes: Synthesis, crystal structures and antiproliferative activity of the first complexes of meclofenamic acid-Novel anti-tuberculosis agents. Journal of Inorganic Biochemistry 103, 738-744.

LUKHOBA, C.W., SIMMONDS, M.S.J., PATON, A.J., 2006. *Plectranthus*: A review of ethnobotanical uses. Journal of Ethnopharmacology 103, 1-24.

LALL, N., MEYER, J.J.M., 1999. In vitro inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. Journal of Ethnopharmacology 66, 347-354.

LALL, N., MEYER, J.J.M., 2000. Antibacterial activity of water and acetone extract of the roots of *Euclea natalensis*. Journal of Ethnopharmacology 72, 313-316.

LALL, N., MEYER, J.J.M., 2001. Inhibition of drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. Journal of

Ethnopharmacology 78, 213-216

MARHWAH, R.G., FATOPE, M.O., DEADMAN, M.L., OCHEL, J.E., AL-SAIDI, S.H., 2007. Antimicrobial activity and the major components of the essential oil of *Plectranthus cylindraceus*. Journal of Applied Microbiology 103**,** 1220-1226.

MATHABE, M.C., HUSSEIN, A.A., NIKOLOVA, R.V., BASSON, A.E., MEYER, J.J.M., LALL, N., 2008. Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana.* Journal of Ethnopharmacology 116, 194-197.

MATIVANDLELA, S.P.N., LALL, N., MEYER, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South African Journal of Botany 72, 232-237.

MATU, E.N., VAN STADEN, J., 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology 87, 35-41

NARUKAWA, Y., SHIMIZU, N., SHIMOTOHNO, K., TAKEDA T., 2001. Two new diterpenoids from *Plectranthus nummularius* Briq. Chemical and Pharmaceutical Bulletin 49, 1182-1184.

NERYA, O., VAYA, J., MUSA, R., IZRAEL, S., BEN-ARIE, R., TAMIR, S., 2003.Glabbrene and Isoliquiritigenin as tyrosinase inhibitors from licorice roots. Journal of Agricultural and Food Chemistry 51, 1201-1207.

NEWTON, S.M., LAU, C., GURCHA, S.S., BESRA, G.S., WRIGHT, C.W., 2002. The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis.* Journal of Ethnopharmacology 79, 57-67.

PUUPPONEN-PIMIÄ, R., NOHYNEK, L., ALAKOMI, H-L., OKSMAN-CALDENTEY, K-M., 2005. Bioactive berry compounds- novel tools against human pathogens. Applied Microbiology and Biotechnology 67, 8-18.

RABE, T., VAN STADEN, J., 1998., Screening of Plectranthus species for antibacterial activity. South African Journal of Botany 64, 62-68.

SALIE, F., EAGLEAS, P.F.K., LENG, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27-33.

SIMÕES, M.F., et al., (In press) An easy and and stereo selective rearrangement of an abietane diterenoid into a bioactive microstegiol derivative. Phyochemistry Letters (2010). doi:10.1016/j.phytol.2010.09.001

VÄLIMAA, A-L, HONKALAMPI-HÄMÄLÄINEN, U., PIETERINEN, S., WILFÖR, S., HOLMBOM, B., VON WRIGHT, A., 2007. Antimicrobial and cytotoxicity knotwood extracts and related pure compounds and their effects on food-associated microorganisms. International Journal of Food Microbiology 115, 235-243.

VAN DER KOOY, F., MEYER, J.J.M., LALL, N., 2006. Antimycobacterial activity and possible mode of action of newly isolated neodiospyrin and other naphthoquinones from *Euclea natalensis*. South African Journal of Botany 72, 349-352.

ZENTZ, F.,LE GUILLOU, R., LABIA, R., SIROT, D., LINARD, B., VALLA, A., 2004. Syntheses, in vitro antibacterial and cytotoxic activities of a series of 3- substituted succinimides. IL FARMACO 59, 879–888.