CHAPTER 7

Extraction and isolation of antifungal compounds

7.1. Introduction

Plants are a good source of compounds that can be used for medicinal and other purposes because they protected themselves against fungal attack by synthesizing chemical compounds. However among the thousands of compounds present only a small number of these can be used. Thus it is time consuming and often difficult to use classical methods of extraction and complete separation of individual compounds and then test these isolated compounds for biological activities. The more practical route is to screen plant extracts for a specific biological activity, select the promising compounds and purify them further. In this bioassay guided fractionation and column chromatography were mainly used. From results obtained in previous chapters *C. nelsonii* was selected for isolation of antifungal compounds. *C. nelsonii* was also chosen because it contained more active compounds had high activity against all tested pathogens and it was active at low concentrations.

7.2. Materials and methods

7.2.1. Extraction procedure

A number of factors were taken into consideration in selecting solvents that were to be used in the serial exhaustive extraction (**Section 2.1**). The choice of solvent also depended on what was planned with the extract. The effect of solvent on subsequent bioassay was an important factor.

The *Combretum nelsonii* leaves were carefully examined and old, insect damaged, fungus-infected leaves were removed. Healthy leaves were dried at room temperature. Once the leaves were dry, they were ground to a fine powder of c. 1.0 mm diameter. Material was stored in a closed container at room temperature.

The defatting process by hexane is important in the isolation process since non-polar compounds will be extracted fast in this process. Hence, serial exhaustive extraction was used on *C. nelsonii* leaf powder with hexane as a starting solvent followed by dichloromethane (DCM), acetone and methanol as extractants. The polarity of solvents gradually increased and ranged from a non-polar solvent (hexane) to a more polar solvent (methanol). This was to ensure that a wide polarity range of compounds could be extracted in the process.

Dried powdered leaves (502 g) of *C. nelsonii* were exhaustively extracted in a serial manner with solvents of increasing polarity. Finely ground plant material (502 g) was initially extracted in a Labotec model 20.2 shaking machine at high speed with 5 litres of hexane. The solvent was allowed to extract for 1 hour while shaking. Rotarvapour was used to concentrate the extracts. The solvent was recovered and reused for the next extraction. before being decanted. The same quantity of solvent was added to the marc and shaken once again for an hour. The process was repeated three times. The marc was allowed to dry and the process of extraction was repeated three times with dichloromethane, then acetone, and finally methanol.

The extracts were vacuum filtered through Whatman (no. 2) filter paper using a Buchner funnel, and most of the solvent was removed by vacuum distillation in a Buchi rotary evaporator at 60^0C. Once concentrated to a small volume, the extracts were placed in pre-weighed beakers and allowed to dry completely in front of a cool stream of air. The mass extracted with each solvent was calculated. To determine chemical profile by TLC, 20 mg of each extract was weighed into a pill vial and made up to a concentration of 10 mg/ml by re-dissolving in acetone.

7.2.2. Analysis by TLC

The chemical profile of extracts was determined by TLC using aluminum backed thin layer chromatography plates (Merck, silica gel 60 F_{254}). The following three solvent systems were used to develop the plates: EMW, CEF and BEA (**Section 2.1.5**).

7.2.3. Bioautography

Bioautography was done according to Begue and Kline (1972) with modifications as explained in **Section 6.1.2** and fungal test organisms (**Chapter 5**) were used.

7.2.4. Microdilution assay

The serial dilution microplate dilution (Eloff, 1998) method was used to determine the Minimum Inhibitory Concentration (MIC) values of the extracts against each test fungal species with modifications as explained in **Chapter 5**.

7.2.5. Total activity

Total activity in ml/mgindicates the degree to which the active extracts, fractions or compounds in one gram of plant material can be diluted and still inhibit the growth of the test organisms (Eloff, 2000). This makes it possible to quantify the efficiency of fractionation and determine loss or gain of activity (Eloff, 2004). It was calculated as explained in **Chapter 5**.

7.2.6. Isolation

Since the acetone and DCM fractions of *C. nelsonii* had a high number of antifungal compounds they were subjected to column chromatography starting with the acetone fraction.

7.2.6.1. Open column chromatography

Column chromatography was used to further simplify the acetone fraction from serial exhaustive extraction. The acetone fraction from *C. nelsonii* was dried in a rotary evaporator to determine the mass of the fraction to be used for column chromatography.

The dry method for packing of chromatographic columns was used; silica gel 60 was poured slowly into a column (15.5 cm x 10 cm), on top of a small amount of cotton wool. The dry sample of acetone fraction (12.38 g) of *C. nelsonii* was then placed neatly on top of the silica in the column. Filter paper cut to the internal diameter of the column and cotton-wool were neatly placed on top of the sample to prevent disturbance at the surface during solvent introduction. Fifteen elution systems were added slowly in the order as in **Table 7.1**. With the addition of solvent (1.2 L) into the column, the vacuum was switched on. The solvent was allowed to run through the column; until the 1.2 L had been collected in the beakers through a separating funnel. The beakers were allowed to evaporate overnight under a cool stream of air and TLC analysis was then carried out.

Table 7.1. Solvent mixtures used in column chromatography

Elution system	
Hexane:	100 %
Hexane: Ethyl acetate	90 %
	80 %
	70 %
	50 %
	30 %
	10 %
Ethyl acetate	100%
Ethyl acetate: Methanol	90 %
	80 %
	70 %
	60 %
	50 %
	40 %
Methanol	100%

The composition of each fraction was analysed using TLC.

7.2.6.1. Analysis and grouping of fractions

After vacuum liquid chromatography, beakers were placed under a stream of air to facilitate concentration of the fractions for TLC analysis and bioassays. After about 50% of the volume of the eluent had been evaporated, the volume was measured and 5 ml was collected from each beaker into a pre-weighed pill vial and allowed under a stream of air to dry rapidly. The mass of each fraction was calculated and the concentration (10 mg/ml) determined. Fractions were analysed by TLC (**Chapter 2**).

7.2.6.2. Combination of fractions

From TLC results, fractions were combined, based on the similarity of their chemical profile. Combined fractions were placed under an air current to facilitate drying and crystallization. Once dry, the fractions were weighed to calculate the total mass fractionated and the crystallized fractions were washed with a combination of solvents to obtain pure compounds. Active fractions were combined and subjected to further column chromatography.

In order to select the best mobile phase for eluting the 80% ethyl acetate fraction and 90% ethyl acetate fractions, 5 µl of a 10 mg/ml (i.e. 50 µg) solution was placed in a narrow band c. 1 cm wide on TLC plates and developed with various combination of solvents. The solvent that exhibited the most favourable separation of compounds was chosen.

7.3. Results of Vacuum Liquid Chromatography

7.3.1. Extraction

Finely ground, dried *C. nelsonii* leaves (502 g) were serially extracted with hexane, DCM, acetone and methanol as indicated above. The following masses in **Table 7.2.** were obtained.

Table 7.2. The mass (g) of *C. nelsonii* leaf powder serially extracted with four extractants from 502 g.

Extractants		**Mass residue extracted (g)**		
		Mass	**Total**	**Total activity**
Hexane	I	4.29	6.93	99
	II	1.88		
	III	0.76		
DCM	I	9.32	16.29	407.25
	II	3.66		
	III	3.31		
Acetone	I	10.59	12.38	213
	II	1.31		
	III	0.48		
Methanol	I	28.35	40.58	676.3
	II	8.11		
	III	4.12		
TOTAL			**76.18**	**1395.55**

The total mass extracted was 76.18 g from 502 g of *C. nelsonii*. Methanol (40.58 g) extracted the highest mass from *C. nelsonii*, followed by DCM (16.29 g); acetone (12.38 g) and hexane (6.93 g) extracted the lowest mass. Success in isolating compounds from plant material is largely dependent on the type of the solvent used in the extraction procedure (Lin *et al*., 1999). The total mass extracted using different solvents (acetone, hexane, DCM and methanol) are shown in **Figure 7.1.** Methanol was the best extractant, extracting a greater quantity of plant material than any of the other solvents. The important factor is actually not quantity, but the biological activity.

7.3.2. Phytochemical analysis

The separated compounds on TLC chromatograms were made visible by spraying with vanillin-sulphuric acid and heating at 105 ^{o}C (**Figure 7.2**). The BEA separation system had

number of compounds followed by CEF, and EMW had the least number of compounds, which means more polar compounds were separated. The DCM extract contained more compounds in CEF and EMW as compared to other extracts. Greatest separation was noticed in CEF.

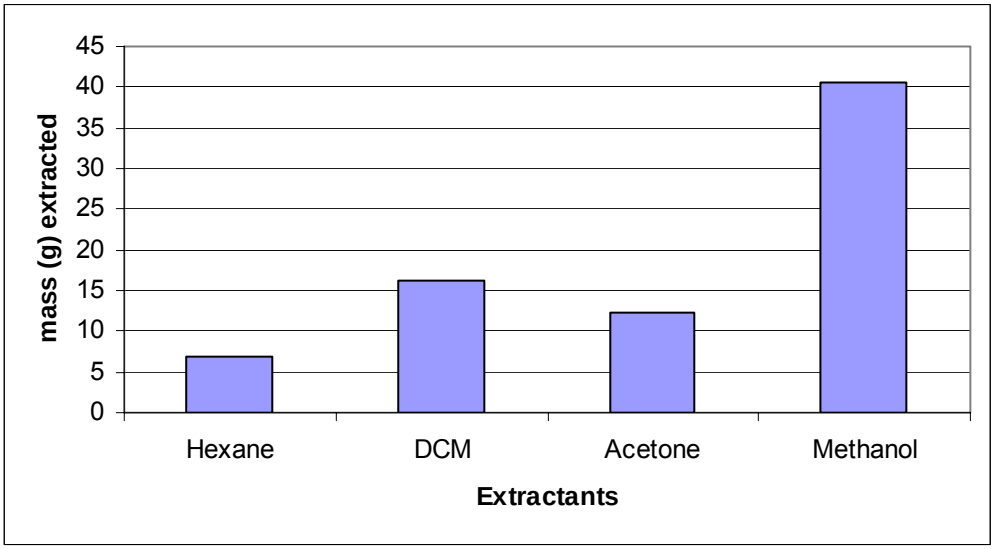

Figure 7.1. Mass serially extracted by hexane, DCM, acetone, and methanol from *C. nelsonii*

7.3.3. Quantitative antifungal activity

All of the extracts had substantial antifungal activity against different pathogens tested (**Table 7.3**). *C. neoformans* and *M. canis* were the most sensitive microorganisms with an average MIC value of 0.02 mg/ml, followed by *C. albicans* (0.04 mg/ml). The least sensitive were *A. fumigatus* and *S. schenckii* with average MIC's of 0.09 and 0.10 mg/ml respectively (**Table 7.3**).

Table 7.3. Minimum Inhibitory Concentration (MIC) of *C. nelsonii* extracts after 24 H.

Microorganisms	MIC values (mg/ml)												
	Hexane			DCM			Acetone			Methanol			Average
	I	II	III	I	II	III	I	II	III	I	II	III	
C. albicans	0.02	0.04	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.08	0.08	0.08	**0.04**
C. neoformans	0.04	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	**0.02**
A. fumigatus	0.16	0.16	0.16	0.04	0.04	0.04	0.08	0.08	0.16	0.04	0.08	0.08	**0.09**
M. canis	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	**0.02**
S. schenckii	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.16	0.16	0.02	0.16	0.16	**0.10**
Average	**0.06**	**0.06**	**0.07**	**0.04**	**0.04**	**0.04**	**0.04**	**0.06**	**0.08**	**0.04**	**0.07**	**0.07**	
Total Average	**0.07**			**0.04**			**0.06**			**0.06**			

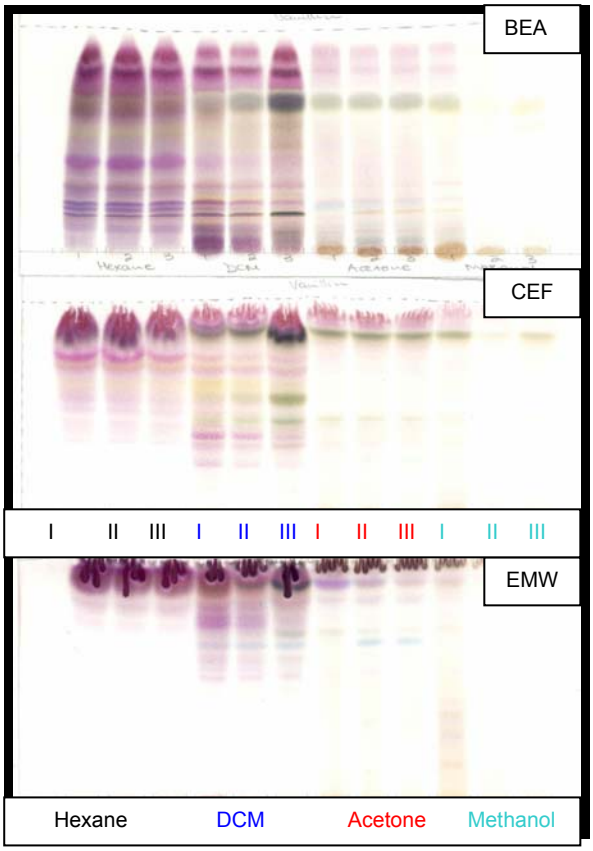

Figure 7.2. Chromatograms of *C. nelsonii* extracts developed in BEA (top), CEF (centre), and EMW (bottom) solvent systems and sprayed with vanillin–sulphuric acid to show compounds extracted with acetone, DCM, hexane and methanol.

DCM extracts had the highest average antifungal activity with the average MIC of 0.04 mg/ml, followed by acetone (0.06 mg/ml) and methanol (0.06 mg/ml), then hexane (0.07 mg/ml).

Total activity was also calculated. The reason for this is explained in **Chapter 5.** Not only the MIC but also the quantity in fraction is important. Extracts with higher values are most promising to work with. All extracts had substantial total activity against *C neoformans* and *M. canis*, with average of 307 and 317 ml/g respectively, followed by *C. albicans* with 185 ml/g (**Table 7.4**). *S. schenckii* and A*. fumigatus* were less sensitive with average total activity of 161 and 122 ml/g respectively. Methanol extracts had the highest average total activity (461 ml/g) and hexane the lowest with 65 ml/g. The first methanol extract had the highest average total activity of 1063 ml/g and the lowest was the third acetone extract with 16 ml/g.

Table 7.4. Total activity in ml/g of *C. nelsonii* extracts after 24 hours incubation at 37 °C.

Microorganisms	Total activity (ml/g)												Average
	Hexane			DCM			Acetone			Methanol			
	I	II	III	I	II	III	I	II	III	I	II	III	
C. albicans	215	47	19	466	183	166	530	66	24	354	101	52	**185**
C. neoformans	107	94	19	466	183	166	530	66	24	1418	406	206	**307**
A. fumigatus	27	12	5	233	92	83	132	16	3	709	101	52	**122**
M. canis	215	94	38	466	183	166	530	66	24	1418	406	206	**317**
S. schenckii	54	24	10	117	46	41	132	8	3	1418	51	26	**161**
Average	**123**	**54**	**18**	**350**	**137**	**124**	**371**	**44**	**16**	**1063**	**213**	**108**	
Total Average	**65**			**204**			**143**			**461**			

Methanol had the highest total activity and that looks promising but the difficult part is to remove methanol from the extracts and chemistry of polar compounds are difficult to work with.

7.3.4. Quantitative analysis of antifungal compounds

The extracts were analysed by bioautography for quantitative analysis of antifungal compounds on the chromatograms. Chromatograms were sprayed with *C. albicans* (**Figure 7.3a**), *C. neoformans* (**Figure 7.3b**), *S. schenckii* (**Figure 7.4a**), *A. fumigatus* (**Figure 7.4b**) and *M. canis* (**Figure 7.4c**).

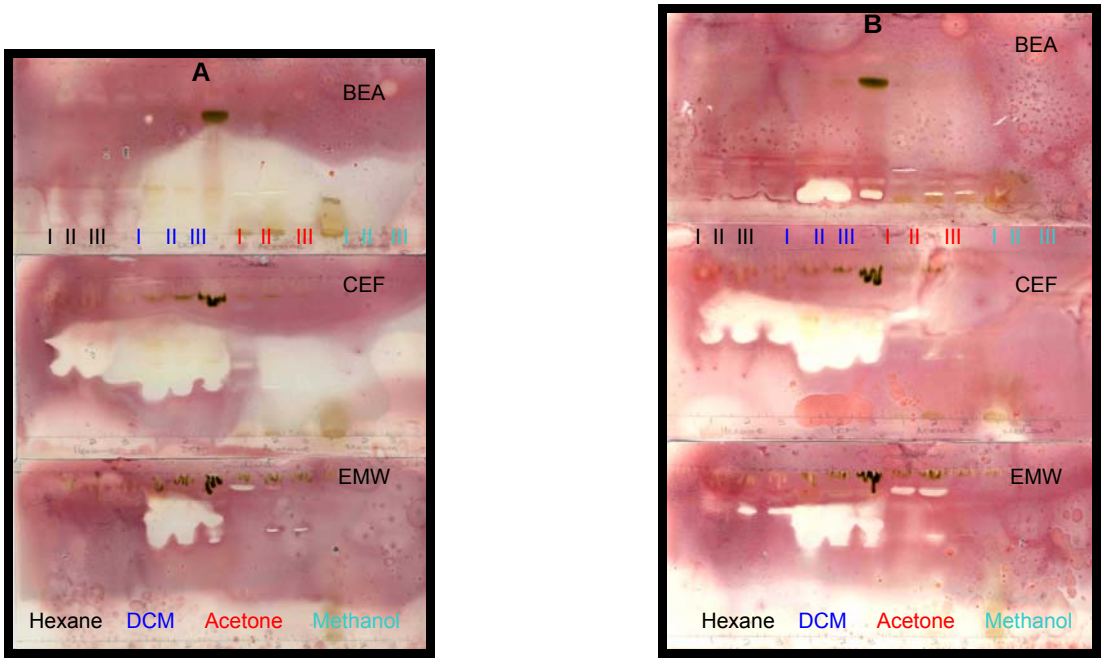

Figure 7.3. Bioautography of *C. nelsonii* extracts separated by BEA (top), CEF (Centre) and EMW (Bottom) and sprayed with *C. albicans* (A) and *C. neoformans* (B). White areas indicate active compounds that inhibited the growth. (I, first extraction; II, second extraction; III, third extraction).

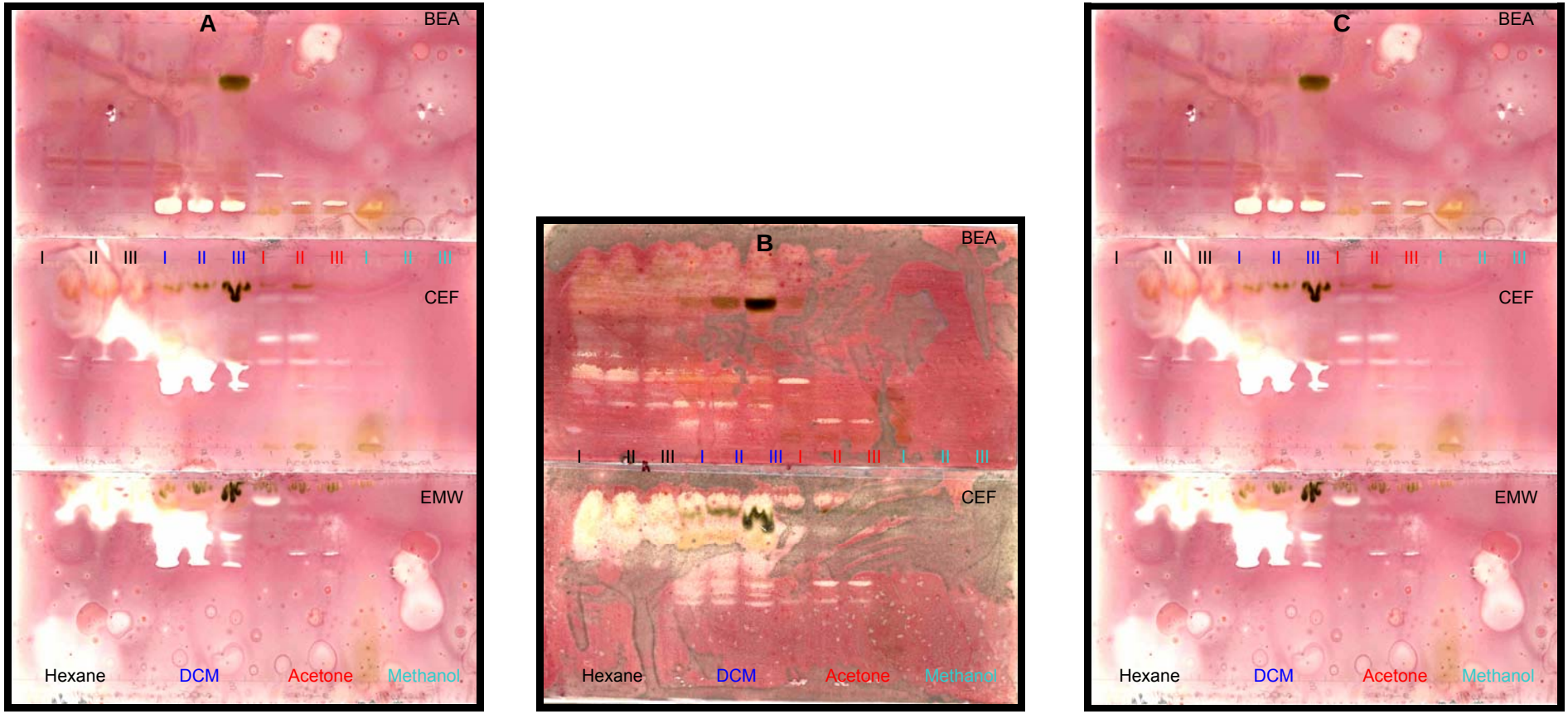

Figure 7.4. Bioautography of *C. nelsonii* extracts separated by BEA (top), CEF (Centre) and EMW (Bottom) and sprayed with *S. schenckii* (A), *A. fumigatus* (B) and *M. canis* (C). White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth. (I, first extraction; II, second extraction; III, third extraction).

DCM extracts of *C. nelsonii* contain very active compounds against all tested microorganisms, followed by acetone extracts. The CEF separation system separated active compounds against all organisms, and it will be used in the next experiments. BEA separated active compounds only in DCM extracts. Hexane extracts had more activity against *C. albicans* and *C. neoformans* in CEF only, and for *S. schenckii* and *M. canis* in both CEF and EMW. Methanol extracts didn't have any activity and were discarded. Acetone and DCM extracts were used for column chromatography because they had more active compounds. It was decided not to use the hexane extracts as they were oily and had lower activity than the acetone and DCM. When leaves were extracted with methanol alone, there was activity, but when methanol was used in serial exhaustive extraction the was no activity. It might be due synergistic effects of compounds in the cruse extract.

7.3.5. Fractionation of VLC fractions

Acetone extracts (12.38 g) of *C. nelsonii* were combined and subjected to the column (23 cm X 3 cm). Isolation was done as indicated in **Section 7.2**; several eluents were used (**Table 7.1**). The masses of all fractions were recorded (**Table 7.5**). Hexane: Ethyl acetate (80:20) and Ethyl acetate: Methanol (90:10) fractions had the highest masses of 1.119 and 1.524 g respectively. The lowest mass was in the Ethyl acetate: methanol (40:60), 0.130 g fraction. DCM fractions will be discussed in the later stages.

Table 7.5. The mass (g) of *C. nelsonii* acetone and DCM extracts fractions recovered by VLC with different eluents.

Eluent	Percentages (%)	Mass (g)	
		Acetone	DCM
Hexane:	100%	0.347	0.243
Hexane: Ethyl acetate	90%	0.868	3.065
	80%	1.119	1.624
	70%	0.842	0.715
	50%	0.607	2.252
	30%	0.690	1.808
	10%	0.303	0.589
Ethyl acetate	100%	0.204	0.253
Ethyl acetate: Methanol	90%	1.524	1.250
	80%	0.964	1.024
	70%	0.568	0.930
	60%	0.334	0.687
	50%	0.189	0.437
	40%	0.130	0.128
Methanol	100%	0.190	0.360
Total		**8.879**	**15.365**

Out of the 12.38 g of *C. nelsonii* acetone extract used, I managed to collect 8.88 g using different eluent systems. All the plates were separated with CEF because more active compounds were found in CEF separation (**Figure 7.3** to **7.4**). Phytochemical analysis of the isolates was done (**Figure 7.5**). Bioautography was done on all isolates to locate active compounds (**Figure 7.6** to **7.9**).

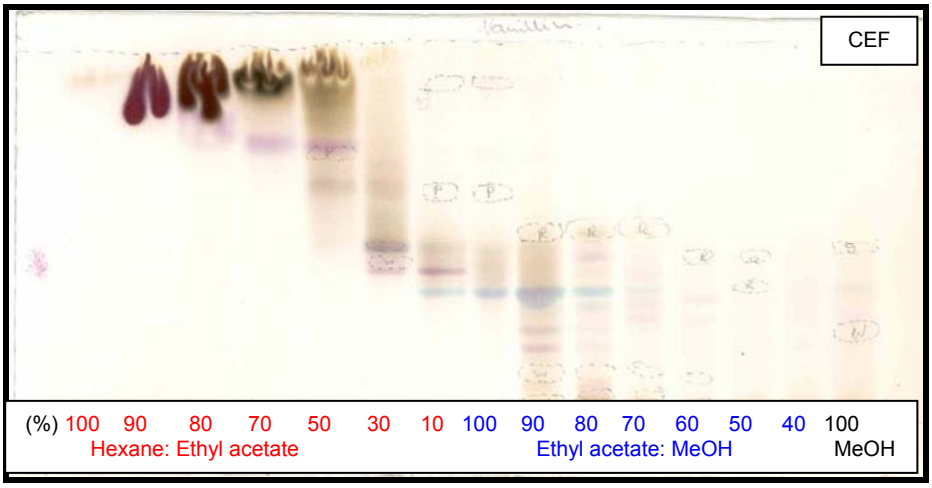

Figure 7.5. Chromatograms of *C. nelsonii* acetone extracts developed in CEF solvent systems and sprayed with vanillin–sulphuric acid to show compounds isolated with different eluent systems.

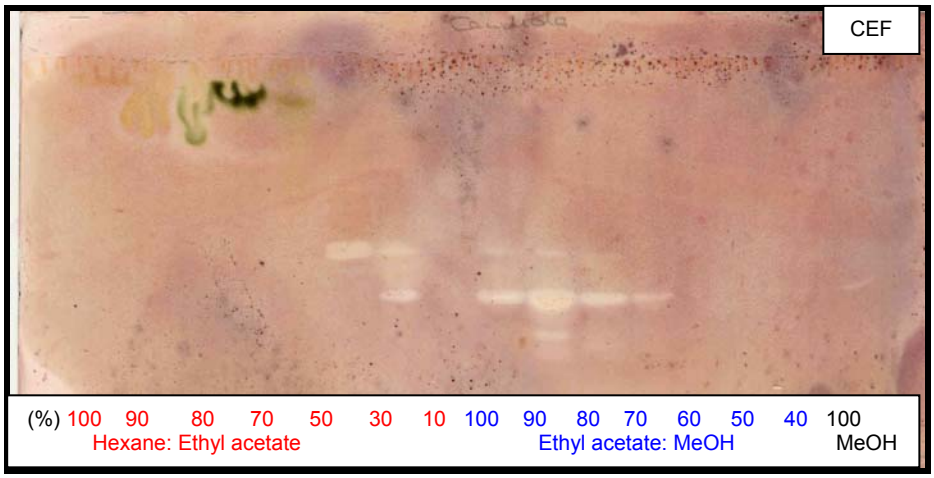

Figure 7.6. Bioautography of *C. nelsonii* acetone extracts separated by CEF and sprayed with *C. albicans.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *C. albicans.*

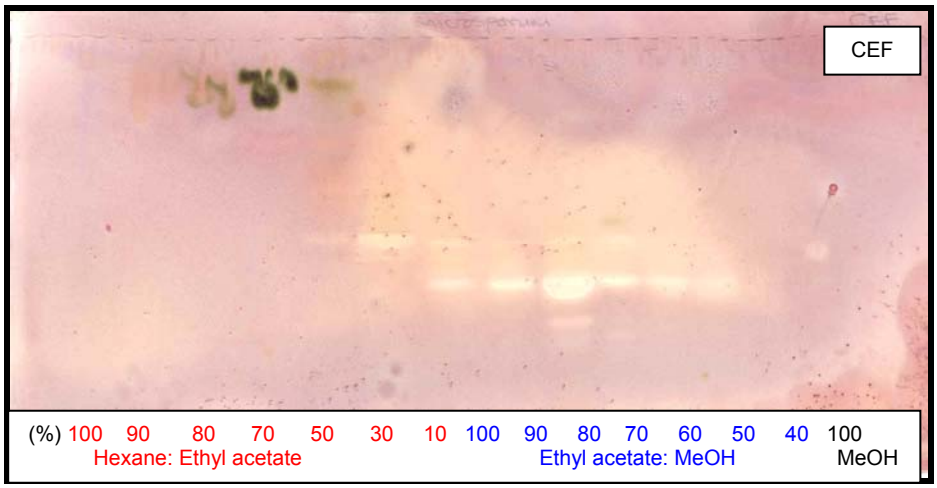

Figure 7.7. Bioautography of *C. nelsonii* acetone extracts separated by CEF and sprayed with *M. canis.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *M. canis.*

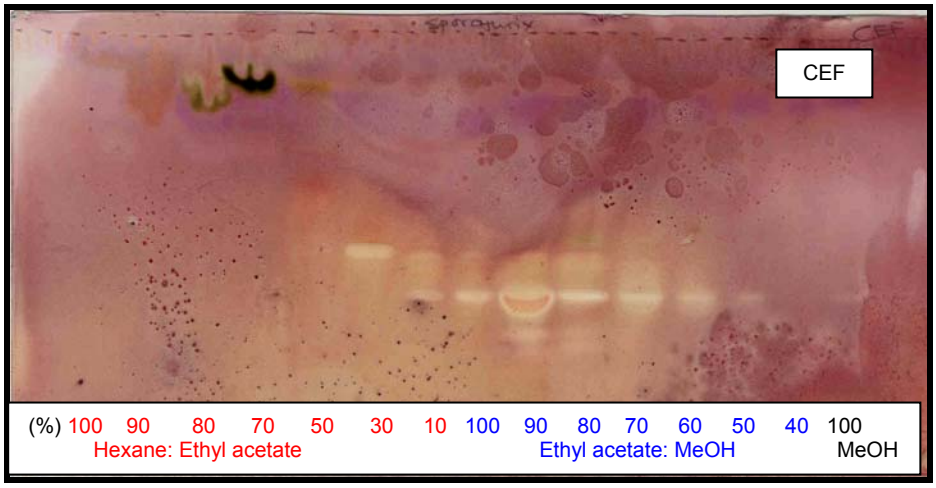

Figure 7.8. Bioautography of *C. nelsonii* acetone extracts separated by CEF and sprayed with *S. schenckii.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *S. schenckii.*

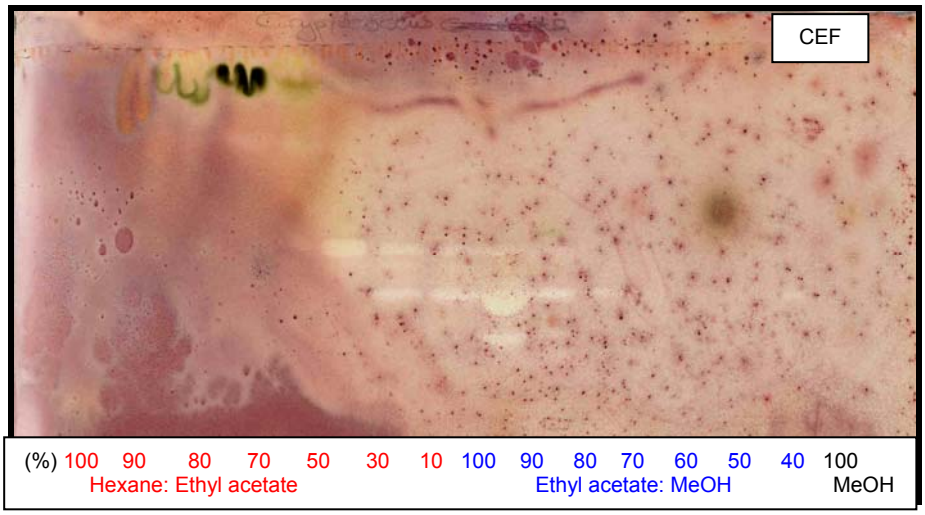

Figure 7.9. Bioautography of *C. nelsonii* acetone extracts separated by CEF and sprayed with *C. neoformans.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *C. neoformans.*

Antifungal compounds against the four fungi tested were present in 50 to 10% hexane in ethyl acetate and 100 to 70% ethyl acetate in methanol fractions (**Figures 7.6** to **7.9**). *A. fumigatus* results were not clear although there was inhibition (results not presented). Only the active compounds in **Figure 7.6** to **7.9** were blue after treating with the vanillin spray reagent (**Figure 7.5**). The most active compound was located in 90% ethyl acetate in methanol fraction. In the following experiments the blue compound was consequently followed for isolation.

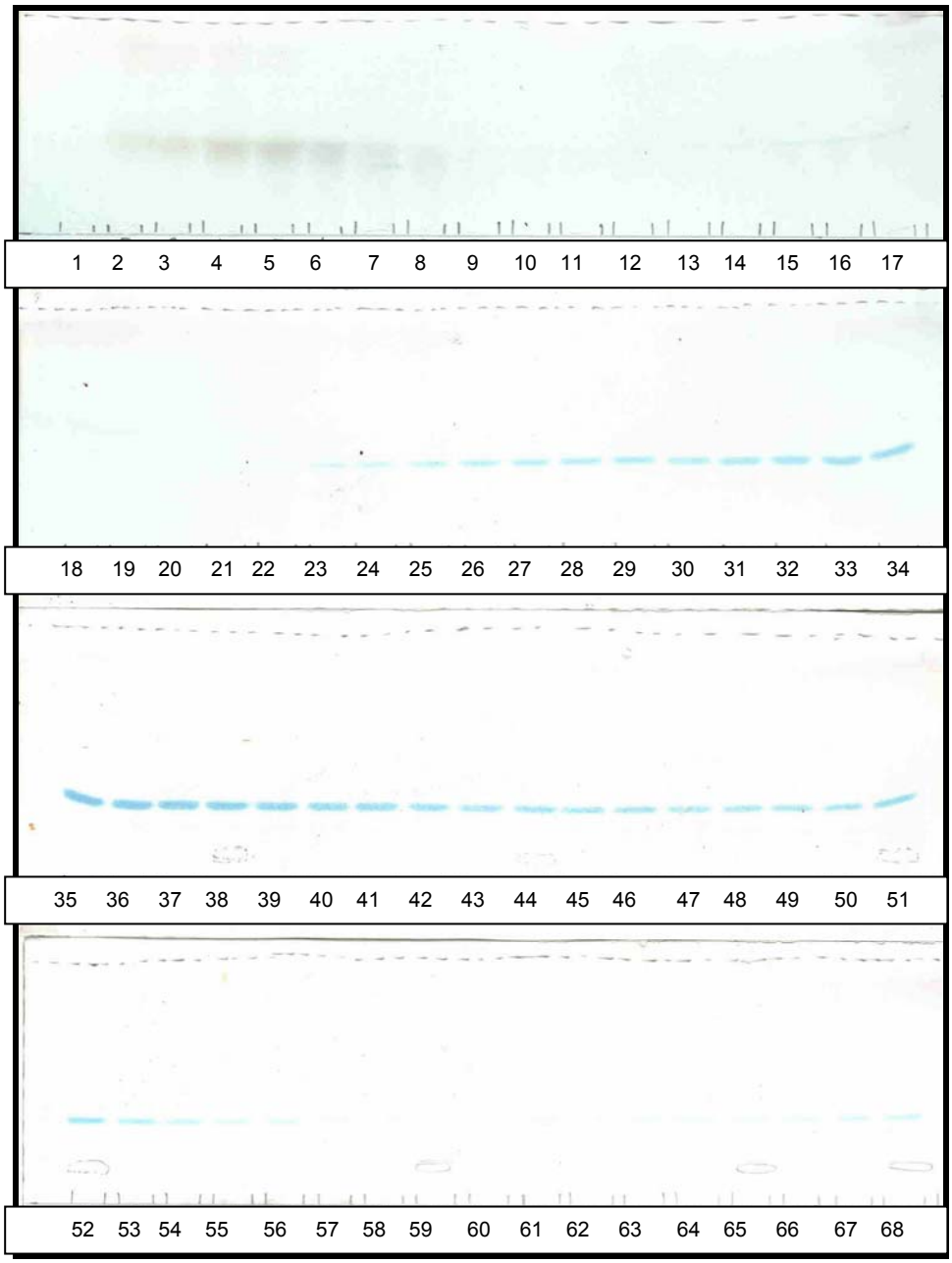

Figure 7.10. Chromatograms of *C. nelsonii* acetone extracts isolated with 90% ethyl acetate and developed in CEF solvent systems and sprayed with vanillin–sulphuric acid to show compounds isolated with different eluent systems.

The fractions from 90 and 80% ethyl acetate in methanol were combined. The masses were 1.524 and 0.964 g respectively, resulting in a total of 2.488 g. After evaluating several eluent systems by TLC, 90% ethyl acetate in hexane was the best, in separating compounds.

Column was prepared as in **Section 7.2.6.1.** Different fractions were collected as indicated in **figure 7.10**. Fractions were combined **Section 7.2.6.2**. Fractions were combined as indicated in the flow chart of the overview of isolation of active compounds (**figure 7.11**).

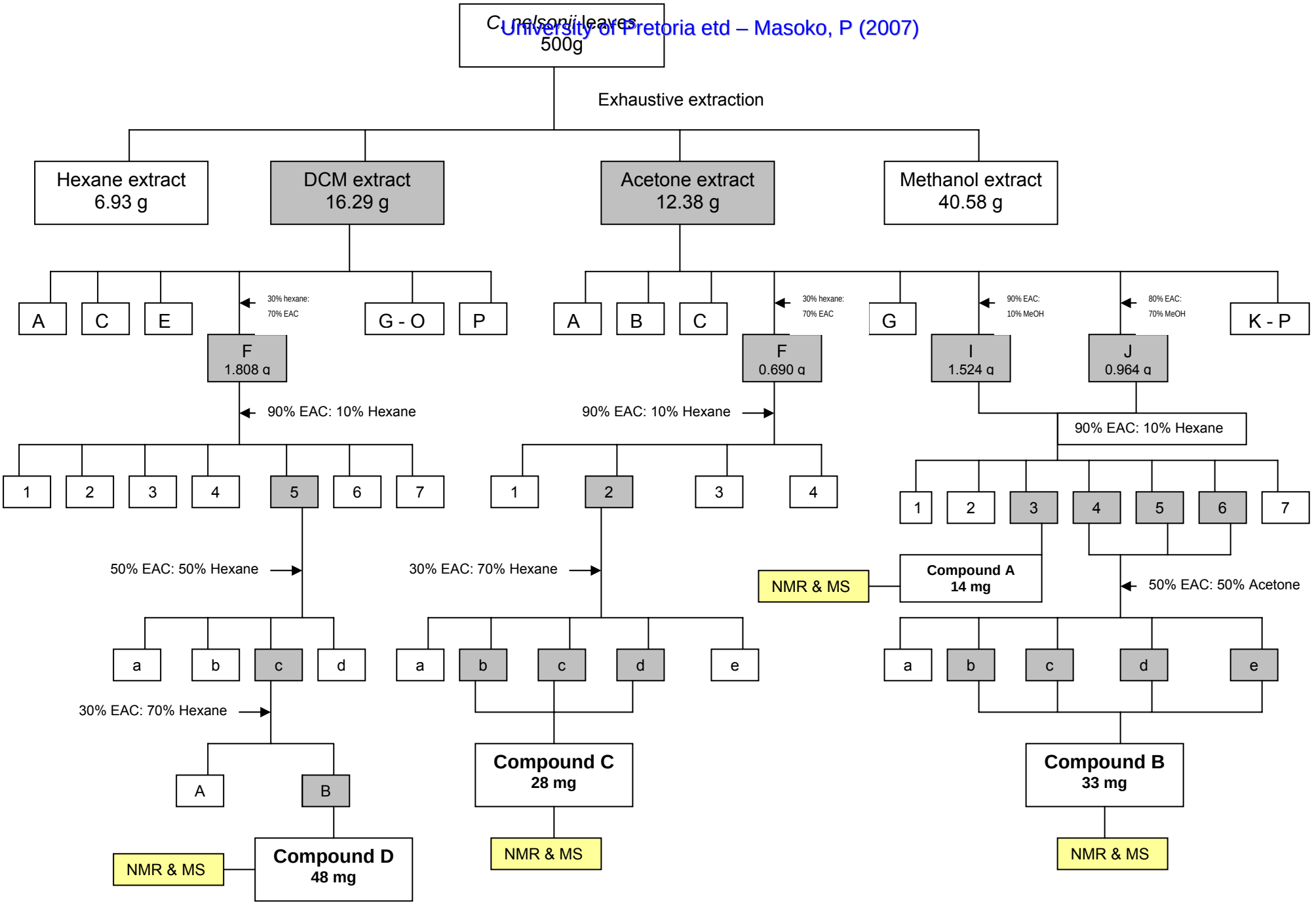

Figure 7.11. Overview of isolation process of four active compounds

7.3.6. DCM Fractions

DCM extracts (21.45 g) from the serial extraction were combined and separated on a Si gel column (12 X 10 cm) by eluting (**Section 7.2.6.1**) with different solvents (**Table 7.1**). The different masses were recorded in **Table 7.5**.

From the 21.45 g of *C. nelsonii* DCM extract used, I managed to collect 15.237 g using different eluent systems. All the plates were separated with CEF because more active compounds were found in CEF separation (**Figure 7.2** to **7.6**). The fractions were analyzed by TLC (**Figure 7.12**). Bioautography was done on all isolates to locate active compounds (**Figure 7.13** to **7.17**).

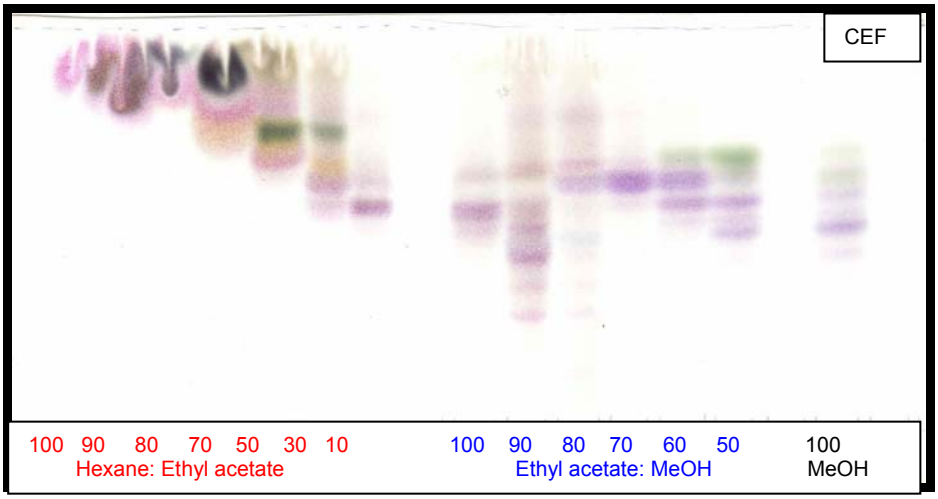

Figure 7.12. Chromatograms of *C. nelsonii* DCM extracts developed in CEF solvent systems and sprayed with vanillin–sulphuric acid to show compounds separated with different eluent systems.

Figure 7.13. Bioautography of *C. nelsonii* DCM extracts separated by CEF and sprayed with *A. fumigatus.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *A. fumigatus.*

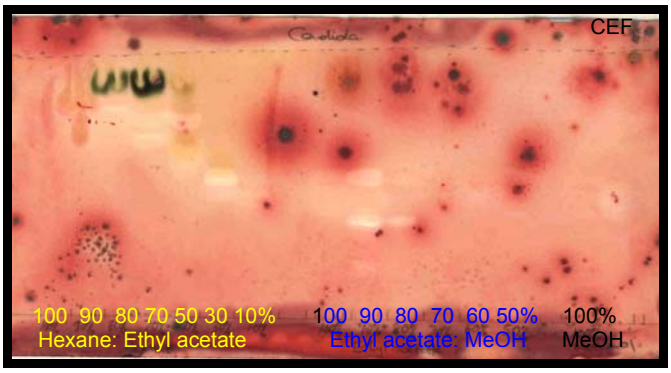

Figure 7.14. Bioautography of *C. nelsonii* DCM extracts separated by CEF and sprayed with *C. albicans.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *C. albicans.*

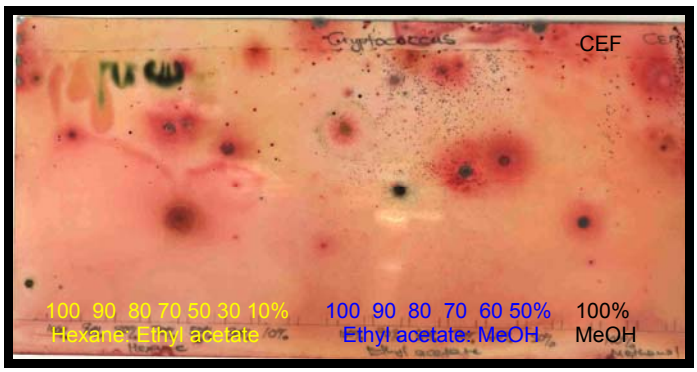

Figure 7.15. Bioautography of *C. nelsonii* DCM extracts separated by CEF and sprayed with *C. neoformans.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *C. neoformans.*

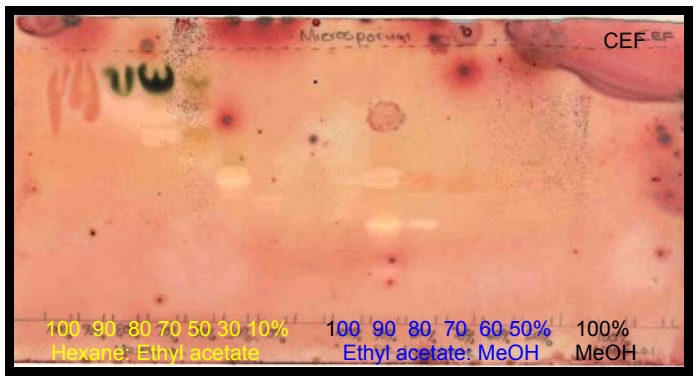

Figure 7.16. Bioautography of *C. nelsonii* DCM extracts separated by CEF and sprayed with *M. canis.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *M. canis*

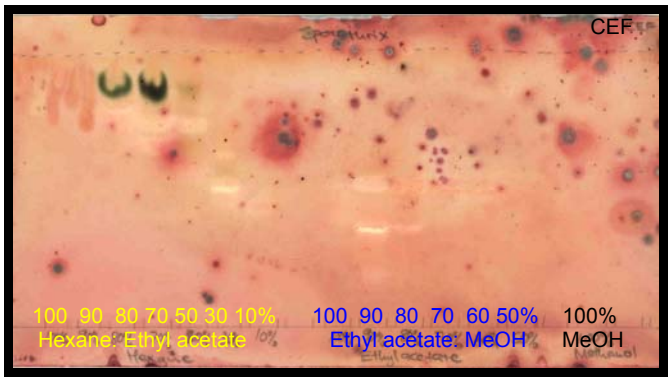

Figure 7.17. Bioautography of *C. nelsonii* DCM extracts separated by CEF and sprayed with *S. schenckii.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of *S. schenckii.*

Active compounds were found in fractions eluted with 80, 70, 50 and 30% hexane in ethyl acetate, and in 90 and 80% ethyl acetate in methanol in *C. albicans, C. neoformans, M. canis* and *S. schenckii*, but in *A. fumigatus* they were found in fractions eluted by 90, 80, 70% ethyl acetate in methanol. Ethyl acetate (90 and 80%) in methanol fractions were combined and subjected to a column and eluted with 90% ethyl acetate in hexane (**Figure 7.18**). The following fractions were combined based on similarity of chromatograms. An overview of isolation is presented in **figure 7.11**. the blue compounds after isolation were again the most active against the fungi tested.

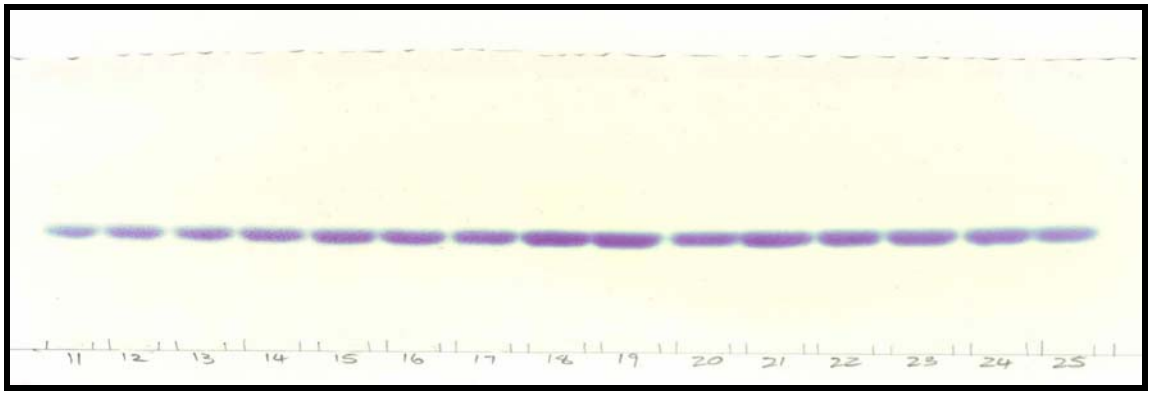

Figure 7.18. Chromatograms of combined fractions of *C. nelsonii* DCM extracts isolated with 80% ethyl acetate in methanol and developed in CEF solvent systems and sprayed with vanillin–sulphuric acid to indicate composition of fractions.

7.4. Discussion and Conclusion

Bioassay-guided fractionation on silica gel 60 (63-200 um) in column chromatography resulted in the successful isolation of the major antifungal compounds present in the leaves of *C. nelsonii*.

A number of active compounds have been isolated from different *Combretum* species. Most of the work so far has been done on antibacterial compounds. The stilbene 2, 3, 4-trihydroxyl, 3, 5, 4-trimethoxybibenzyl (combretastatin B5) was isolated from the leaves of *C. woodii*. It had significant activity against *S. aureus* with an MIC of 16 μg/ml [*Ps. aeruginosa (125* μg/ml), *E. faecalis* (125 μg/ml) and slight activity against *E. coli*.] (Eloff *et al*., 2005a,b). A variety of triterpenoids have been isolated from *Combretum* spp. (Rogers and Verotta, 1996). Terpenes or terpenoids are active against bacteria and fungi (Taylor *et al.*, 1996). Flavonoids isolated from the leaves of *Combretum micranthum* have been shown to have antimicrobial activity against both Gram-positive and Gram-negative microorganisms (Rogers and Verrotta, 1996).

Anti-inflammatory and molluscicidal compounds such as mollic acid –D – glycoside and imberbic acid have been isolated from *C. molle* and *C. imberbe* respectively (Pegel and Rogers, 1985). The saponin, jessic acid linked to α-L-arabinose has been isolated from *Combretum eleagnoides* leaves (Osborne and Pegel, 1984). Chemical studies of the *Combretum* genus have yielded acidic triterpenoids and their glycosides, phenanthrenes, amino acids and stilbenes (Pellizzoni *et al*., 1993). A series of closely related bibenzyls, stilbenes and phenanthrenes have been isolated from *C. caffrum* (Petit *et al*., 1995).

Martini *et al*., (2004a) isolated and characterized seven antibacterial compounds. Four were flavanols: kaemferol, rhamnocitrin, rhamnazin, quercitin 5,3 -dimethylether] and three flavones apigenin, genkwanin and 5-hydroxy-7,4'-dimethoxyflavone.
All test compounds had good activity against *Vibrio cholerae* and *E. faecalis*, with MIC values in the range of 25-50 μg/ml. Rhamnocitrin and quercetin-5,3-dimethylether showed additional good activity (25 μg/ml) against *Micrococcus luteus* and *Shigella sonnei*. Toxicity testing showed little or no toxicity towards human lymphocytes with the exception of 5-hydroxy-7,4-dimethoxyflavone (Martini *et al*., 2004b). This compound is potentially toxic to human cells and exhibited the poorest antioxidant activity. Both rhamnocitrin and rhamnazin exhibited strong antioxidant activity with potential anti-inflammatory activity. Although these flavonoids are known, this was the first report of biological activity with some of these compounds.

Serage (2003) isolated and elucidated the structures of two flavanones alpinetin, pinocembrin, and one chalcone flavokawain-from the leaves of *C. apiculatum subsp apiculatum*. All the compounds had substantial activity against the bacterial pathogens tested.

Angeh (2005) isolated 8 compounds with antibacterial activity from *Combretum* section, Hypocrateropsis. Two new pentacyclic triterpenoids (1α, 24β-dihydroxyl-12-oleanen-29-oic acid-24β-O-α-L-2, 4-diacetylramnopyranoside and 1α, 23β-dihydroxyl-12-oleanen-29-oic acid-23β-O- α-L-4-acetylramnopyranoside) and six known triterpenoids (1α, 3β-dihydroxyoleanen-12-29-oic, 3-hydroxyl-12-olean-30-oic, 3, 30-dihydroxyl-12-oleanen-22-one, 1,3, 24-trihydroxyl-12-olean-29-oic acid, (1α, 22β-dihydroxyl-12-oleanen-30-oic acid) and (24-ethylcholesta-7, 22,25-trien-3-ol-O-β-D-glucopyranoside). All eight compounds had moderate (MIC of 60 µg/ml) to strong (10 µg/ml) antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Mycobacterium vaccae*.

In present study I managed to isolate compounds with antifungal activity from *Combretum nelsonii*. This provides a scientific base for the use of this plant in folk medicine and could be the basis of the novel antifungal.

The structure elucidation of the isolated compound will be dealt with in the next chapter.