Chapter 5

The antibacterial activity of different extracts of selected South African plant species

5.1. Introduction

The most widely and frequently used chemotherapeutic agents are antibiotics. Many such agents currently in use to treat bacterial and parasitic infections were first isolated from natural sources including ethnomedicinal plants (Coe and Anderson, 1996). The development of these drugs presented a huge breakthrough in the management of infections caused by bacteria with extraordinary clinical efficacy. However, the successes of these drugs over the decades have been compromised due to development of resistant strains of these pathogens with a commensurate negative impact on the treatment of disease. With the huge threat posed by these bacterial pathogens and the need to develop potent and less toxic alternatives to existing drugs, natural plant extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for new antibacterial agents.

In most developing countries, the incidence of resistance development in humans is common amongst immunocompromised patients. Most important amongst these, on a global scale, are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus* species, and members of the Enterobacteriaceae, producing plasmid-mediated extended spectrum b-lactamase (ESbL), *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* (Medeiros, 1997; Sajduda *et al.,* 1998). The United States Office for Technology Assessment for instance in 1992 estimated that the minimal hospital costs of 5 types of nosocomial infection acquired due to surgical wound infection and pneumonia that were resistant to antibiotics amounted to US$4.5 billion per year while the direct cost of managing antibiotic resistance in the United States was US$100 million to US$10 billion per year (Levy, 1992; US Office of Technology Assessment, 1995). This widespread emergence of resistant strains remains a clinical dilemma in hospitalized patients and raises a serious concern about the future of an antimicrobial approach. This has lead over the decades to the formulation of strategies by various countries to improve on the rational use of antibiotics in humans, aimed at reducing and eventually eliminating the use of antibiotics for purposes other than in human medicine and the treatment of infection in animals. The strategies further

seek to address the problem of spread of antibiotic-resistant organisms by improving hygienic practices and creating appropriate facilities in both hospital and public health settings (Swann Committee, 1969; Jungkind *et. al.,* 1995; US Office of Technology Assessment, 1995; Witte, 1998; Nikiforuk, 1996; Schwartz *et. al.,* 1997; Plotkin and Kimball, 1997; Government Official Report no. 132, Sweden, 1997; Williams and Heyman, 1998).

To solve the problems associated with the prevailing trend of resistance development, pharmaceutical companies have been involved in synthesizing derivatives of existing chemical classes to obtain drugs with 'expanded' spectrums of antibacterial activity. Besides this approach, current efforts in the development of new agents have made little headway with only two novel agents (a cyclic lipopeptide and an oxazolidinone) and a new streptogramin combination reaching clinical availability in the recent past (Shlaes, 2003). The biological cost associated with development of antimicrobial resistance compounds the problem (Gillespie and McHugh, 1997). Amidst these concerns, phytochemicals with inhibitory activity against b-lactamase-producing Gram-negative bacteria have been investigated (Yam *et al*., 1998), as well as those with inhibition of multidrug resistant (MDR) efflux pumps in *S. aureus* (Stermitz *et al*., 2001) and anti-antibiotic resistance properties (Lee *et al.,* 1998). Similarly the use of some plant phytochemicals in conjunction with conventional antibiotics is seen to potentiate the activity of some antibiotics (Zhao *et al*., 2001; Aqil *et al*., 2005). A recent report, by McGaw *et. al*. (2008) further illustrated the potential of some plants with activity against *Mycobacterium* infections.

Plants are considered to contain biologically active constituents with a wider safety margin than synthetic products (Davis, 1994) and developing countries have a wide diversity of flora with enormous potential that may provide solutions to many of the current resistance problems. This study was therefore aimed at the identification of antibacterial constituents in the selected species of plant chosen for the study (Table 4.1). Water is mostly used in folk remedies for extraction, but this solvent does not extract a wide range of active constituents contained in plants and antibacterial compounds (Eloff, 1998c, Kotze and Eloff). To target polar and non-polar constituents for bioactivity testing in this study, leaves of each selected plant species were extracted using hexane, DCM, acetone and methanol separately and tested for selective activity of the test on pathogens. The selectivity index (SI) is the ratio of the biological activity of the substance to the toxicity. The numbers of different antibacterial compounds present in different extract were determined by bioautography.

5.2. Materials and Methods

5.2.1. Thin layer chromatography (TLC) analysis of crude extracts

The plants were collected and extracted using solvents of varying polarity as described in sections 3.1 and 3.2. Extracted plant materials were spotted onto TLC plates and eluted in different solvent systems as described in section 3.4 for separation of constituents in the different extracts.

5.2.2. Bioautography on TLC plates

Thin layer chromatographic plates were prepared for the assay as described in section 3.4 without spraying with vanillin-sulphuric acid reagent, left to dry for 5 days and sprayed with test pathogens as described in section 3.7.1. Plates sprayed with pathogens were incubated in a humidified atmosphere at 37°C and thereafter sprayed with INT as described (section 3.7.1).

5.2.3. Microdilution assay for MIC determination

The serial microdilution method described by Eloff (1998b) was used to determine the minimum inhibitory concentration of the different plant extracts as described in section 3.7.2. Test pathogens used for the determination of MIC of extracts are described in section 3.7.1. The total activity of plant extracts was determined using the method of Eloff (2004) as described in section 3.8.2.

5.2.3. Determination of cytotoxic effect of the extracts on different cell types

The cytotoxic effect of the different extracts of plants selected for the study was evaluated on Vero, Crandell feline kidney (CRFK) and bovine dermis cells using the method of Mosmann (1983) as described in section 3.9. Selective activity of each extracts was calculated as follows: Selectivity index = CC_{50} / MIC

5.3. Results and Discussion

5.3.1. Chemical constituents of the crude extracts

Chromatograms eluted using CEF gave the best separation of constituents. The diversity of compounds extracted with different extracting solvents of the same plant is presented in Figure 5.1. A slight variation in the chemical composition of different extracts was observed in extracts of the same plant species eluted in CEF. This observation is illustrative of the presence of one or more constituents in different extracts of the same plant, which may serve as possible targets for isolation of biologically active compounds.

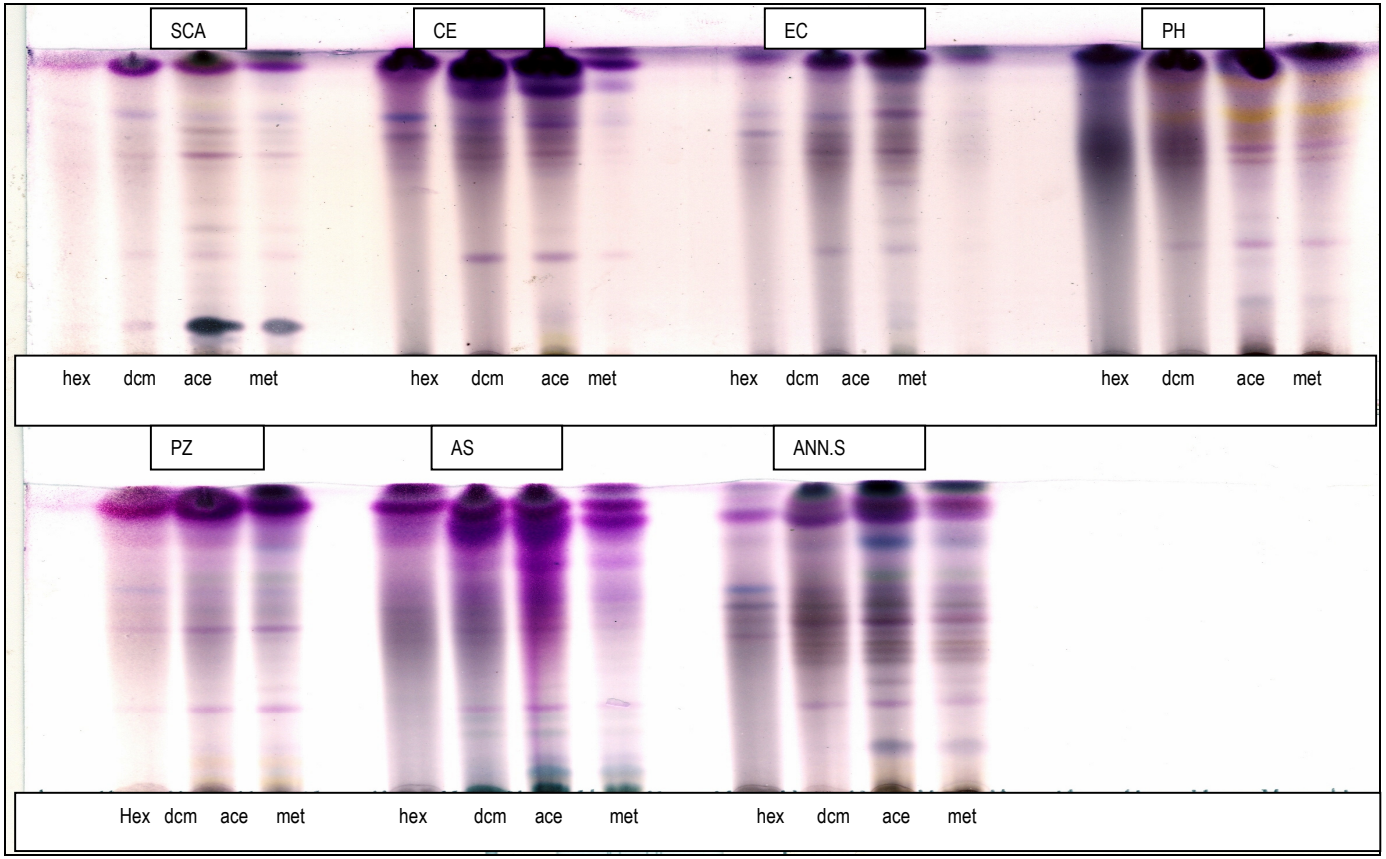

Figure 5.1: Thin layer chromatogram eluted in CEF of separated constituents of the same plant using different solvents for extraction. *SCa = Schrebera alata, CE = Carissa edulis, EC = Ekebergia capensis, PH = Podocarpus henkelii, Pz = Plumbago zeylanica As = Acocanthera shimperi, Ann.s = Annona senegalensis, HEX=Hexane, DCM = Dichloromethane, ACE = Acetone, MET = Methanol*

5.3.2 Inhibition of bacterial growth using bioautography

Bioautography was used to screen for antibacterial compounds to obtain more information on the diversity of compounds present in the different extracts. Inhibitory zones of antibacterial activity were observed as white spots on a purple background following spraying of bioautograms with INT. The R_f values of active constituents were recorded as those white areas on the bioautogram where reduction of INT to a coloured formazan did not occur due to the presence of compounds that inhibit the growth of the test pathogens (Table 5.1). In some cases the growth of the organisms was poor, making it difficult to detect zones of inhibition even when MIC values indicated good antibacterial activity. It is possible that the poor growth resulting in no activity of some extracts may be due to the evaporation or break-down of active compounds during removal of the TLC eluents to the disruption of synergism between active constituents caused by TLC separation or insufficient removal of the TLC eluents (Masoko and Eloff, 2005).

With the mobile systems used, biological activity was observed in all the extracts against one or more organisms. The intermediately polar system (CEF) separated more active constituents, a reflection of the difference in polarity of the systems used. In some cases, the four extracts showed compounds with similar R_f values active against one or more organisms. Bioautograms of *Podocarpus henkelii* extracts for instance displayed active compounds with similar R_f values of 0.21, 0.23, 0.28, 0.26, 0.33 and 0.96 in the different extracts. The extracts were active against *S. aureus* and *E. coli* respectively when eluted in BEA. Another compound, R_f 0.93, also had activity against *S. aureus* and *P. aeruginosa* when eluted in CEF. Likewise, the acetone and methanol extracts of *Acokanthera schimperi* and the dichloromethane extract of *Annona senegalensis* contained compounds active against *S. aureus* with R_f values of 0.72 and 0.88 respectively when eluted in CEF, which were not detected or separated with the other eluent systems.

Table 5.1: Retention factor (R_f) values of active constituents representing zones of inhibition of bacterial growth on bioautograms

Organism	Plant	BEA				CEF				EMW			
		Hex	DCM	Ace	Met	Hex	DCM	Ace	Met	Hex	DCM	Ace	Met
		0.26	0.26	0.26				0.72	0.72	-	-	-	-
	A.S	0.39	0.32	0.32			0.83	0.83	0.83				
		0.46	0.46	0.46									
S. aureus		0.98	0.98	0.98									
	C.E							0.72	0.72				
	Ann.S						0.83	0.83	0.83				
		-	-	-	-	-	0.88			-	-	-	-
	PH	0.21	0.21	0.21	0.21	0.47	0.47	0.47	0.47	0.97	0.97	0.97	0.97
		0.28	0.28	0.28	0.28	0.93	0.93	0.93	0.93				
		0.23	0.23	0.23	0.23		0.90	0.90	0.90	0.97	0.97	0.97	0.97
		0.46				0.93	0.93	0.93	0.93	0.97	0.97	0.97	0.97
	SCa	0.13	0.13	0.13									
	EC	0.26	0.52	0.52									
		0.32											
		-	0.19	0.19	-			-			-		
	PZ		0.26	0.26									
	A.S	-	-	-	-	-	0.88	0.88	0.88	-	0.91	0.91	0.91
	C.E						0.86	0.86	0.86		0.91	0.91	0.91
	Ann.S	-	-	-	-	-	-	-	-		-		
E. coli		0.26	0.26	0.26	0.26	0.91	0.91	0.91	0.91	0.97	0.97	0.97	0.97
	PH	0.33	0.33	0.33	0.33								
		0.96	0.96	0.96	0.96								
	SCa		0.26	0.26	0.26	0.88	0.88	0.88	0.88			0.38	0.38
										0.97	0.97	0.97	
	EC	0.47		0.47		0.91	0.91	0.91	0.91	0.97	0.97	0.97	0.97
	PZ	-	-		-		-				-		
	A.S						0.88	0.88	0.88	-	-	-	-
							0.93	0.93					
	C.E	-	-	-	-		0.84	0.84	0.84	0.97		0.97	
P. aeruginosa	Ann.S						-						
	PH								0.69	-	-	-	-
						0.93	0.93	0.93	0.93				
	SCa	-	-	-	-		0.93	0.93	0.93	-	-	-	-
	EC					0.9							
	PZ						-						

	A.S						0.8	0.8	0.8 0.9	0.92	0.92	0.92	0.92
						0.9		0.9					
	C.E	-	-	-	-		0.8	0.8	0.8	0.92	0.92	0.92	0.92
E. faecalis	Ann.S					0.91	0.91					0.91	0.91
	PH	-	-	-	-	0.95	0.95	0.95	0.95	-	-	0.34	0.34
	SCa					-	0.91	0.91	0.91			0.28	
												0.34	
										0.95	0.95	0.95	0.95
						0.92	0.92	0.92	0.92			0.34	0.34
	EC					0.96	0.96	0.96	0.96	0.92	0.92	0.92	0.92
	PZ	-	-	-	-	-	-	-	-	-	-	-	-

As = Acokanthera schimperi, CE = Carissa edulis, Ann.s = Annona senegalensis, PH = Podocarpus henkelii, SCa = Schrebera alata, Pz = Plumbago zeylanica, EC = Ekebergia capensis

N/A = no activity Hex = Hexane, DCM =Dichloromethane, Ace = Acetone, Met = Methanol

The presence of active zones with similar R_f values may not necessarily indicate the presence of identical compounds in these extracts. More active constituents were identified in extracts eluted in CEF and EMW against all the tested organisms. This suggests the presence of polar and non-polar active compounds in the extracts. The acetone and methanol extracts generally contained more active compounds than the DCM extracts, and hexane extracts showed the lowest number. Comparing the presence of active compounds, variation was observed between the eluent systems, plant species and susceptibility of microorganisms. In plants where BEA was used as eluent, fewer compounds active against *P. aeruginosa* and *E. faecalis* were separated although activity was observed against *S. aureus* and *E. coli* with the same plant extracts eluted in the same solvent system. A possible explanation for this may be that the non-polar and basic nature of BEA could not clearly separate the active non-polar constituents in these plants (Kotze and Eloff, 2002). In most cases, BEA could not move components of extracts from the origin of the TLC plate, indicating the polarity of these compounds. Favourable growth of cultures on bioautograms was observed with *S. aureus* and *E. coli* when compared with the other two microorganisms, which did not grow as well. It is possible that absence of growth may be associated with the sensitivity of these bacteria to residual eluent solvents on the bioautograms (Masoko and Eloff, 2005).

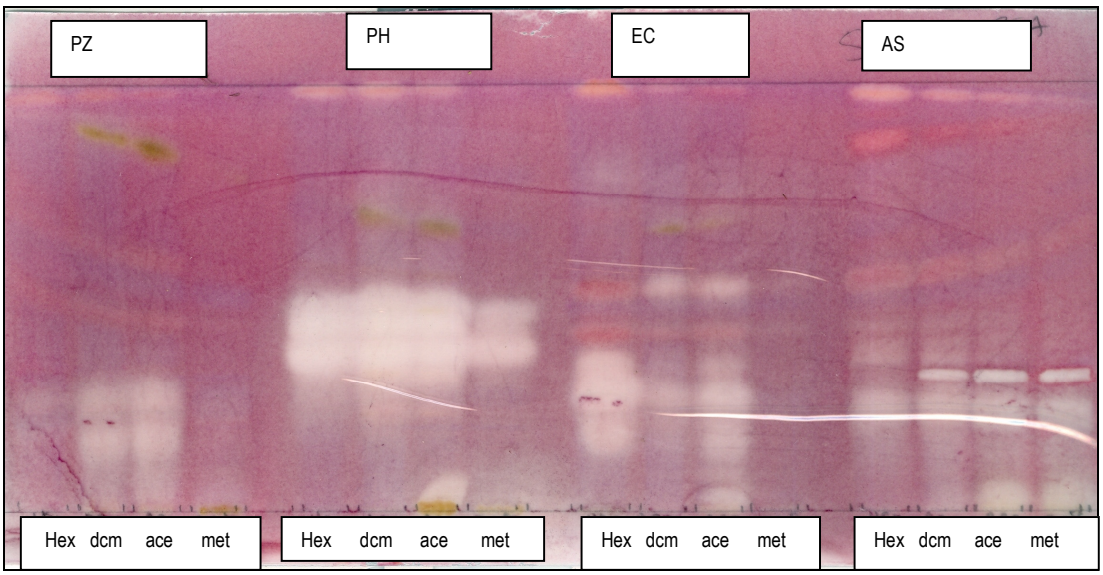

Figure 5.2. A representative bioautograph eluted in BEA indicating inhibition of growth of *S.aureus.*

White zones on a purple background represents inhibition of growth of the pathogen.

PH = Podocarpus henkelii, EC = Ekebergia capensis, As = Acokanthera schimperi,

Pz = Plumbago zeylanica, Hex = Hexane, DCM =Dichloromethane, Ace = Acetone,

Met = Methanol

5.3.3. Antibacterial activity of extracts in terms of MIC values

The MIC values of plant extracts incubated at different time intervals with four representative bacteria are represented in Table 5.2. Studies on South African medicinal plant extracts reporting MIC values below 0.1 mg/mℓ as deserving attention have been published (Eloff, 1999; McGaw *et al.,* 2000; Magee *et al.,* 2007). Thus, in this study, minimum inhibitory concentration values of up to 0.16 mg/mℓ were considered to reflect good antibacterial activity against tested pathogens. The high extraction yield of methanol did not correspond with strong antimicrobial activity except for *Carissa edulis* against *P. aeruginosa*, with MIC = 0.04 mg/mℓ after 24 hours of incubation. Overall, acetone extracts had good activity with MIC values within the range of 0.04 to 0.32 mg/mℓ, DCM 0.02 to 0.64 mg/mℓ and hexane the least after 24 hours of incubation in that order. This observation further supports the relevance of solvent type used in extraction, aimed at targeting bioactive constituents present in plants.

Differences in antibacterial activity were observed with varying times of incubation. The acetone and methanolic extracts of *Carissa edulis* for example, had an MIC of 0.08 mg/mℓ after 12 hours of incubation against *S. aureus*, but after 24 hours, the MIC values increased to 0.16 mg/ml, reflecting a decrease in antibacterial activity. A similar phenomenon was also found among other extracts, with the DCM extract of *Annona senegalensis* against *P. aeruginosa* showing MIC of 0.16 mg/mℓ at 12 hours and 0.02 mg/mℓ at 24 hours incubation. The decrease in MIC from 0.16 to 0.02 mg/mℓ was interesting to note. To investigate the variation in MIC values with time of incubation, contents of wells with MIC = 0.16 mg/mℓ were inoculated on MH agar and incubated overnight but no growth of the organism was apparent. It is possible that prolonged contact time may have resulted in a change in pH, which may be responsible for the observed activity at lower concentrations with this extract. These observations may suggest a possible concentration dependence of crude extracts from static to cidal effects produced *in vitro*.

A variation in susceptibility of Gram-positive and Gram-negative bacterial pathogens was observed between plants and type of extracting solvents (Table 5.3). The observed difference in the degree of susceptibility may be attributed to differences in active constituents extracted by each solvent, the presence of compounds that are acting in consonance with each other or morphological differences that exist between these organisms. Morphologically, the cell wall of Gram-negative bacteria is less permeable to antimicrobial substances than their Gram-positive counterparts (Nostro *et al.*, 2000; Hodges, 2002), which may be responsible for the observed differences in activity

Table 5.2: Antibacterial activity of selected plant species against Gram-positive and Gram-negative bacteria (MIC in mg/mℓ)

Plant species	Parts	Solvents															
		Hexane				Dichloromethane				Acetone				Methanol			
		S.a	*E.c*	*E.f*	*P.a*	*S.a*	*E.c*	*E.f*	*P.a*	*S.a*	*E.c*	*E.f*	*P.a*	*S.a*	*E.c*	*E.f*	*P.a*
Acokanthera schimperi	Leaves	2.2 2.5	1.25 1.25	0.64 0.64	0.64 0.64	0.32 0.32	2.5 2.5	0.16 0.16	0.16 0.16	0.08 0.08	0.08 0.08	0.32 0.32	0.32 0.32	0.16 0.16	0.32 0.32	0.32 0.32	0.64 0.64
Carissa edulis	Leaves	2.5 2.5	2.5 2.5	2.5 2.5	2.5 2.5	0.64 0.16	0.16 0.16	0.32 0.21	0.32 0.04	0.08 0.16	0.32 0.16	0.32 0.16	0.32 0.16	0.08 0.16	0.16 0.16	0.32 0.32	0.64 0.04
Annona senegalensis	Leaves	2.5 2.5	2.5 2.5	2.5 2.5	2.5 2.5	0.16 0.16	0.08 0.04	0.16 0.08	0.16 0.02	0.32 0.32	0.32 0.32	0.32 0.08	0.32 0.04	1.25 0.32	0.64 0.32	0.64 0.64	2.5 0.32
Podocarpus henkelii	Leaves	2.5 2.5	2.5 2.5	2.5 2.5	2.5 2.5	0.32 0.32	0.32 0.32	0.32 0.32	0.32 0.32	0.32 0.32	0.16 0.16	0.32 0.32	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.32 0.32
Schrebera alata	Leaves	2.5 2.5	2.5 2.5	2.5 2.5	2.5 2.5	0.16 0.16	0.32 0.32	0.13 0.13	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16
Ekebergia capensis	Leaves	2.5 2.5	2.5 2.5	2.5 2.5	2.5 2.5	0.13 0.13	0.16 0.16	0.32 0.32	0.32 0.32	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16	0.16 0.16
Plumbago zeylanica	Leaves	0.64 0.64	0.32 0.32	0.16 0.16	2.5 2.5	0.08 0.08	0.08 0.08	0.08 0.08	0.08 0.08	0.16 0.16	0.08 0.08	0.08 0.08	0.08 0.08	0.08 0.08	0.32 0.32	0.16 0.16	0.32 0.32
Gentamicin		0.003	0.003	0.006	0.006												

S.a = *Staphylococcus aureus*, E.c = *Escherichia coli,* E.f = *Enterococcus faecalis*, P.a = *Pseudomonas aeruginosa*

Table 5.3: Total activity values of different plant extracts against bacteria after 24 hours of incubation

Plant species	Solvents															
	Hexane				Dichloromethane				Acetone				Methanol				Average
	S.a	*E.c*	*E.f*	*P.a*	*S.a*	*E.c*	*E.f*	*P.a*	S.a	E.c	E.f	P.a	S.a	E.c	E.f	P.a	
Acocanthera schimperi	12	24	46.88	46.88	164.06	21	328.13	328.13	1312.5	1312.5	328.13	328.13	1562.5	781.25	781.2 5	390.63	485.5
Carissa edulis	9	9	9	9	265.63	265.63	202.38	1062.5	312.5	312.5	312.5	625	1640.6	1640.6	820.31	6562.5	878.7
Annona senegalensis	5	5	5	5	203.75	812.5	406.25	1625	62.5	62.5	250	500	234.38	234.38	117.19	234.38	297.7
Podocarpus henkelii	14	14	14	14	93.75	93.75	93.75	93.75	203.13	406.25	203.13	406.25	1093.75	1093.75	1093.75	546.88	342.3
Schrebera alata	3	3	3	3	156.25	78.13	192.31	156.25	203.13	203.13	203.13	203.13	734.38	734.38	734.38	734.38	271.6
Ekebergia capensis	4	4	4	4	173.08	140.63	70.31	70.31	156.25	156.25	156.25	156.25	578.13	578.13	578.13	578.13	212.9
Plumbago zeylanica	11.72	23.44	46.88	3	156.25	156.25	156.25	156.25	46.88	93.75	93.75	93.75	1406.3	351.56	703.13	351.56	240.6
Average	8.3	11.7	18.3	12.1	173.2	223.9	207.0	498.8	328.1	363.8	220.9	330.3	1035.7	773.4	689.7	1342.6	

Table 5.4: Selectivity index values relating observed activity of extracts with cytotoxicity

	HEXANE					DICHLOROMETHANE					ACETONE					METHANOL				
Plants	CC_{50} ug/ml	S.a	E.c	E.f	P.a	CC_{50}	S.a	E.c	E.f	P.a	CC_{50}	S.a	E.c	E.f	P.a	CC_{50}	S.a	E.c	E.f	P.a
SCA	22.0	0.01	0.01	0.01	0.01	32.0	0.20	0.10	0.25	0.20	30.0	0.19	0.19	0.19	0.19	25.0	0.16	0.16	0.16	0.16
EC	42.0	0.02	0.02	0.02	0.02	27.0	0.21	0.17	0.08	0.08	30.0	0.19	0.19	0.19	0.19	670.0	4.19	4.19	4.19	4.19
PZ	36.0	0.06	0.11	0.23	0.14	42.0	0.53	0.53	0.53	0.53	32.0	0.20	0.40	0.40	0.40	>1000	12.5	4.47	8.94	4.47
PH	56.0	0.02	0.02	0.02	0.02	42.0	0.13	0.13	0.13	0.13	45.0	0.14	0.28	0.14	0.28	42.0	0.26	0.26	0.26	0.13
CE	75.0	0.03	0.03	0.03	0.03	0.4	0.00	0.00	0.00	0.01	>1000	6.25	6.25	6.25	6.25	10.0	0.06	0.06	0.03	0.25
ANN.S	15.0	0.01	0.01	0.01	0.01	27.0	0.17	0.68	0.34	1.35	8.0	0.03	0.03	0.10	0.20	<0.001	0.003	0.003	0.001	0.003
AS	30.0	0.01	0.02	0.05	0.05	0.4	0.00	0.00	0.00	0.00	<0.001	0.01	0.01	0.003	0	<0.001	0.006	0.003	0.003	0.001
Berberine	10	10.00	0.17	0.33	0. 33															

As = *Acocanthera schimperi*, CE = *Carissa edulis*, Ann.s = *Annona senegalensis*, PH = *Podocarpus henkelii*, SCa = *Schrebera alata*, Pz = *Plumbago zeylanica*, EC = *Ekebergia capensis*, S.a = *Staphylococcus aureus*, E.c = *Escherichia coli*, E.f = *Enterococcus faecalis*, P.a = *Pseudomonas aeruginosa*

The quantity of antibacterial constituents present in each extract was determined by calculating total activity values (Table 5.3). This value indicates the volume to which the biologically active constituents originally present in 1 g of dried plant material can be diluted and still be potent enough to kill the microbial pathogen (Eloff, 1999). Here we chose to calculate total activity values from MIC values after 24 hours of incubation, the longest time the pathogens were in contact with plant extracts. From the 112 extracts tested, the bioactivity of the methanol extract of *Carissa edulis* against *P. aeruginosa* gave the highest total activity value 6562, followed by *Acokanthera schimperi* against *S. aureus.* Although MIC values of acetone extracts showed stronger antibacterial activity than methanol extracts, total activity values for methanol extracts were higher than those obtained for acetone extracts. Excellent selectivity index values (Table 5.4) were obtained for the methanol extract of *Plumbago zeylanica* against *S. aureus* and good to moderate activity against *Enterococcus faecalis, E.coli* and *P.aeruginosa*. The acetone extract of *Carissa edulis* on the other hand exhibited excellent SI values against all tested pathogens, while the methanol extract of *E. capensis* showed moderate activity against all the tested pathogens. This indicates that these plants have the highest inhibition of bacterial growth with relatively low toxicity to mammalian cells.

Overall, going by MIC values, acetone extracts of these plant species comparatively had the best activity followed by the DCM and hexane the least. The activity of the acetone extract is consistent with previous report (Eloff, 1999) were acetone is considered as the best extract for antimicrobial activity. However, methanol extracts had the best selectivity index value, followed by acetone and hexane the least. The susceptibility of pathogens was generally highest with methanol extracts of plants against *S.aureus* and *E. faecalis.* Hexane and methanol extracts of two plant species were less toxic , acetone extract, one plant species and DCM extracts none, indicating that constituents of DCM of these plants may generally be metabolic toxin (Table 5.4).

5.4. Conclusion

This study investigated the use of different extracts of the same plant to test for antibacterial activity. The separation of active constituents by TLC and subsequent exhibition of activity on bioautography by some compounds indicates the diversity of constituents present in extracts depending on the solvent used for extraction. Also, the presence of more than one active constituent on bioautography in extracts indicates synergistic effect of components of extracts

on test pathogens. All the plant species tested showed varying degrees of activity, with some having a broad spectrum of activity against test pathogens. Generally, acetone extracts had good antibacterial activity, followed by DCM extracts with MIC values as low as 0.08 mg/mℓ. The use of selectivity index values to determine the relationship of activity of a test substance to its cytotoxic concentration is important, especially where the use of a crude extract with synergistic activities is contemplated. This value can help decide whether the compounds active in an extract are general metabolic toxins. The antibacterial activity observed with most of the extracts according to selectivity index values, except for the methanol extract of *Plumbago zeylanica* against *S. aureus*, *E. faecalis, E. coli* and *P. aeruginosa* and the acetone extract of *Carissa edulis* against all tested pathogens, may be due to the toxic effect of the extracts on test pathogens. Results suggest that the choice of solvent used for extraction of a particular plant species can influence the biological activity of the extract.

In this study, plants with low to moderate toxicity and good total activity value, had antibacterial activity depending on the type of solvent used for extraction. Variation however exists in the susceptibility of bacteria, virus and fungal pathogens to the different extracts of these plant species. In the next chapter, the different extracts of these plants will be evaluated for antiviral activity against selected animal viruses.