CHAPTER 3: RESULTS

3.1 Acetylcholinesterase inhibitory activity

The results of the plant extracts showing moderate to good inhibition of AChE in various solvents, are provided in Figures 3.1 and 3.2. At the highest concentration tested, 50% showed good (>50% inhibition), 45.5% showed moderate (30-50% inhibition) and 4.5% low (<30% inhibition) AChE inhibition. Percentage activity as described by Vinutha et al. (2007) was used. Generally, inhibition was dose-dependent and the higher activity of the organic extracts may suggest that organic solvents are able to extract more active compounds with possible AChE inhibitory activity than water. No false positive reactions were observed with the assay on the TLC plates (results not shown).

The IC_{50} values of the plant extracts indicating AChE inhibitory activity are presented in Table 3.1. Ethyl acetate extracts of the roots of *C. bulbispermum*, *X. undulatum*, *L. schweinfurthii*, *S. puniceus* and bulbs of *B. disticha* all had very low IC_{50} values. Although the IC_{50} of these plant extracts were higher than that of galanthamine (0.00053 mg/ml), they possess good AChE inhibitory activity considering they are still mixtures containing various compounds.

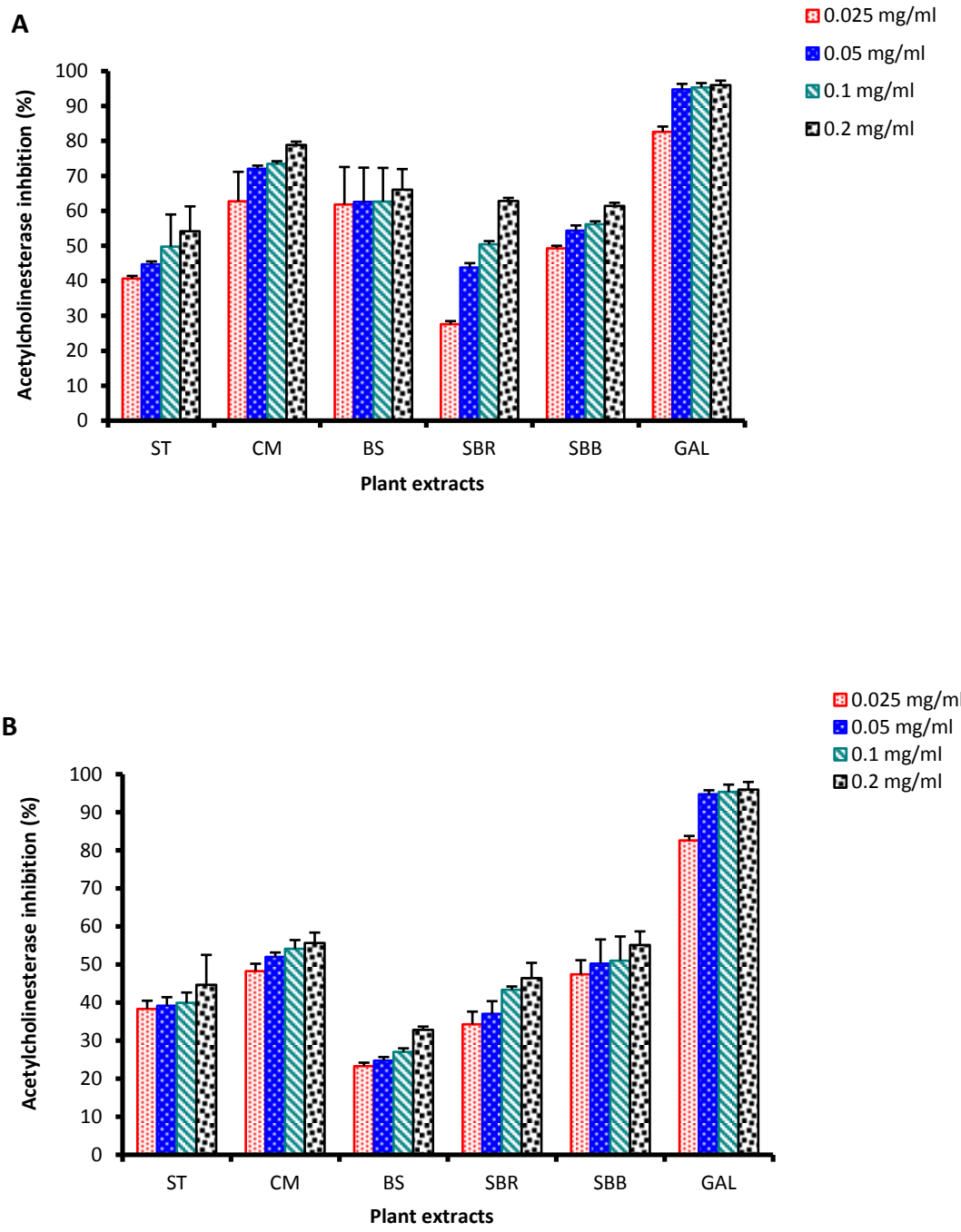


Figure 3.1 AChE inhibitory activity (%) of (A) DCM: MeOH (1:1) extracts and (B) water extracts of plants with moderate to good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *Schotia brachypetala* (bark); GAL, Galanthamine (positive control).

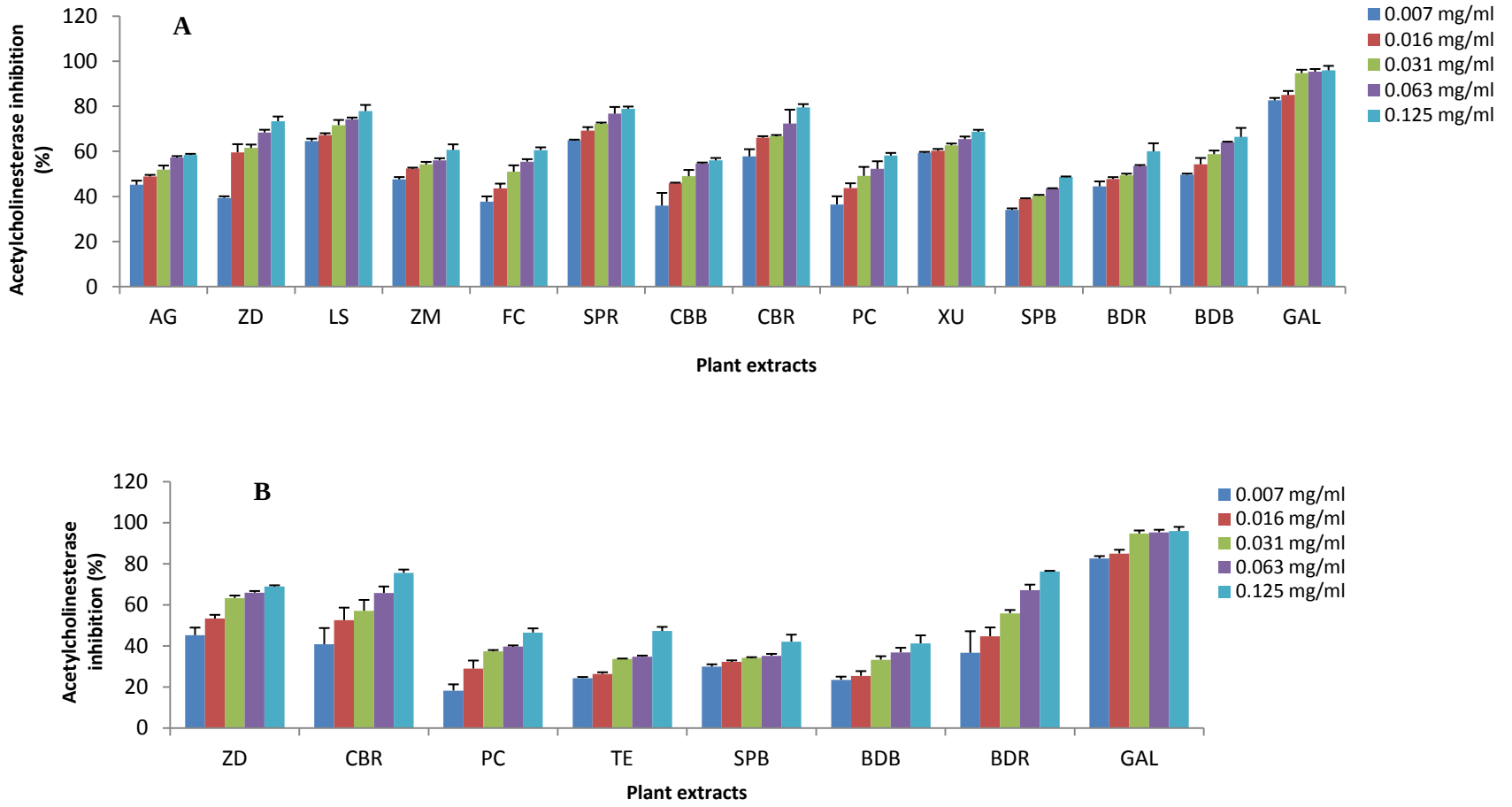


Figure 3.2 AChE inhibitory activity (%) of (A) ethyl acetate extracts and (B) methanol extracts, of plants with moderate to good activity. AG, *Adenia gummifera* (root); ZD, *Zanthoxylum davyi* (root); LS, *Lannea schweinfurthii* (root); ZM, *Ziziphus mucronata* (root); FC, *Ficus capensis* (fruit); SPR, *Scadoxus puniceus* (root); SPB, *S. puniceus* (bulb); CBB, *Crinum bulbispermum* (bulb); CBR, *C. bulbispermum* (root); PC, *Piper capense* (root); XU, *Xysmalobium undulatum* (root); BDR, *Boophane disticha* (root); BDB, *B. disticha* (bulb); *Tabanaemontana elegans* (root); GAL, Galanthamine (positive control).

Table 3.1 Acetylcholinesterase inhibitory activity of the plant extracts as represented by their IC_{50} values.

Species	Plant part analyzed	Extraction solvent	AChE inhibition IC_{50} (mg/ml)
Salvia tiliifolia	Whole plant	DCM:MeOH (1:1)	1.0000 ± 0.010
		Water	12.0000 ± 1.200
Chamaecrista mimosoides	Root	DCM:MeOH (1:1)	0.0300 ± 0.080
		Water	0.3500 ± 0.020
Buddleja salviifolia	Whole plant	DCM:MeOH (1:1)	0.0500 ± 0.020
		Water	*
Schotia brachypetala	Root	DCM:MeOH (1:1)	0.8900 ± 0.010
		Water	3.4000 ± 0.500
	Bark	DCM:MeOH (1:1)	0.2700 ± 0.070
		Water	0.4900 ± 0.040
Adenia gummifera	Root	Methanol	*
		Ethyl acetate	0.0189 ± 0.005
Piper capense	Root	Methanol	*
		Ethyl acetate	0.0407 ± 0.012
Zanthoxylum davyi	Root	Methanol	0.0100 ± 0.004
		Ethyl acetate	0.0116 ± 0.002
Xysmalobium undulatum	Root	Methanol	*
		Ethyl acetate	0.0050 ± 0.000
Lannea schweinfurthii	Root	Methanol	*
		Ethyl acetate	0.0030 ± 0.000
Terminalia sericea	Root	Methanol	*
		Ethyl acetate	*
Ziziphus mucronata	Root	Methanol	*
		Ethyl acetate	0.0112 ± 0.003
Tabernaemontana elegans	Root	Methanol	*
		Ethyl acetate	*
Ficus capensis	Fruits	Methanol	*
		Ethyl acetate	0.0319 ± 0.005
Scadoxus puniceus	Bulb	Methanol	*
		Ethyl acetate	*
	Root	Methanol	*
		Ethyl acetate	0.0030 ± 0.000
Crinum bulbispermum	Bulb	Methanol	*
		Ethyl acetate	0.0393 ± 0.014
	Root	Methanol	0.0148 ± 0.039
		Ethyl acetate	0.0021 ± 0.007
Boophane disticha	Bulb	Methanol	*
		Ethyl acetate	0.0073 ± 0.002

	Root	Methanol	0.0199 ± 0.009
		Ethyl acetate	0.0230 ± 0.007
Tulbaghia violacea	Bulb	Methanol	*
		Ethyl acetate	*
	Root	Methanol	*
		Ethyl acetate	*
Control			
Galanthamine			$5.3 \times 10^{-4} \pm 1.0 \times 10^{-5}$

* represents extracts with maximum inhibition below 50% at the highest concentration tested

3.2 Antioxidant activity and polyphenolic content

The dose-dependent ABTS and DPPH radical scavenging activity of the plant extracts with good antioxidant activity is depicted in Figures 3.3 and 3.4, respectively. Antioxidant activity is expressed as a percentage of the ratio of the decrease in absorbance of the test solution to that of ABTS or DPPH solution without the plant extracts. The plants with good activity showed a propensity to quench the free radicals, as indicated by the dose-dependent increase in percentage inhibition. The ethyl acetate extracts of all the plants with the exception of *T. sericea* showed either no activity or very low radical scavenging activity in both the DPPH and ABTS assays. This may indicate that solvents of high polarity are able to extract more antioxidant compounds than intermediate polar solvents, for the plants investigated in the study.

The IC_{50} values (concentration of the extract that is able to scavenge half of the DPPH or ABTS radical) are presented in Table 3.2. The DCM: MeOH (1:1) extracts of the root of *S. brachypetala* and the methanol extract of the root of *L. schweinfurthii* and *T. sericea* showed the highest radical scavenging activity.

The plant extracts which showed good antioxidant activity (>50%), in both assays, were further screened for determination of the level of total phenols and flavonoids (Table 3.3). All these extracts contained phenols, with the highest amounts in the DCM: MeOH (1:1) and water extracts of *S. brachypetala* root and bark, and methanol extract of *P. capense* root. The plant extracts also contained some flavonoids with the highest found in methanol extracts of *T. sericea* roots.

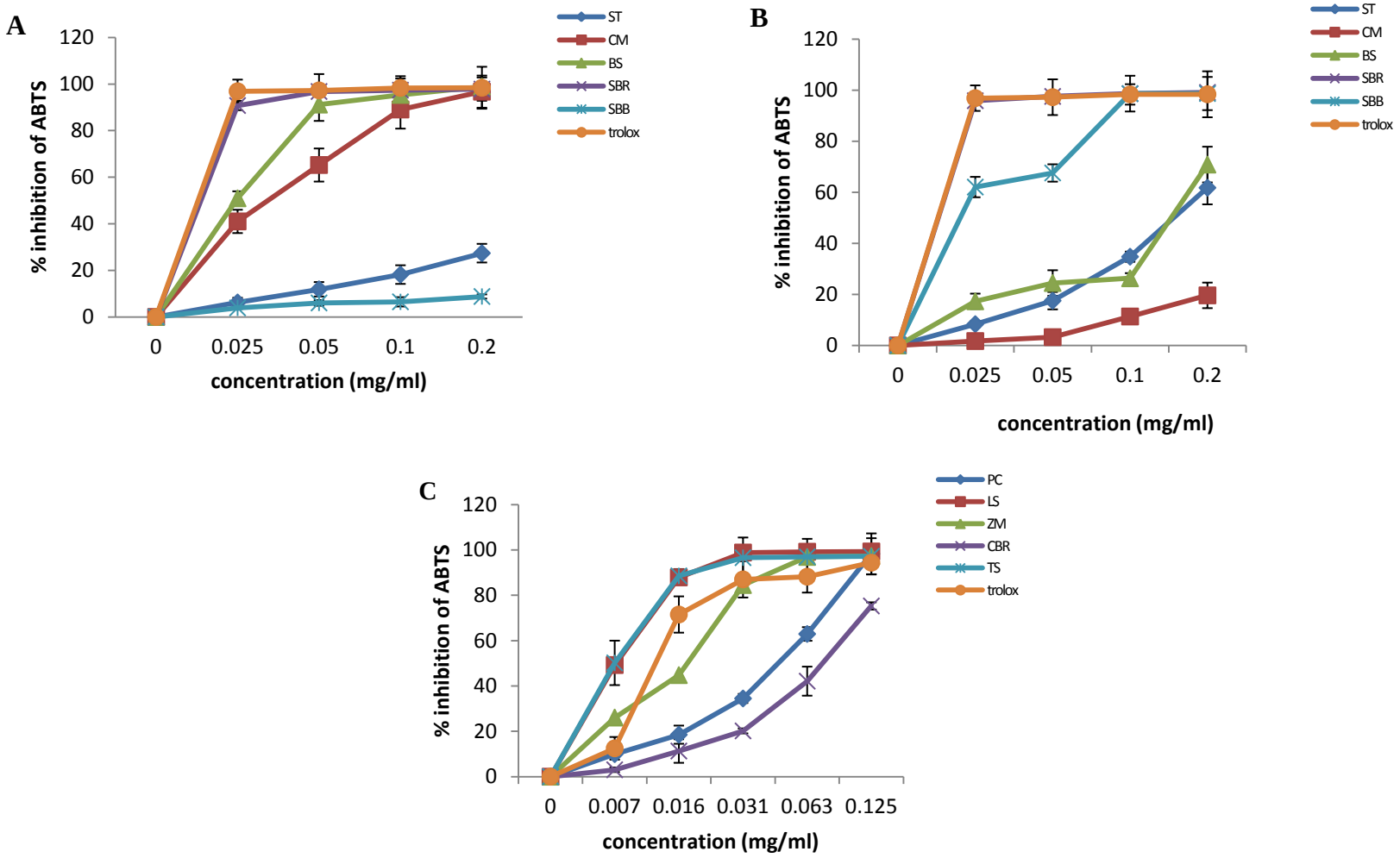


Figure 3.3 ABTS radical scavenging activity of (A) DCM: MeOH (1:1) extracts, (B) water extracts, and (C) methanol extracts, of plants with good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *S. brachypetala* (bark); PC, *Piper capense* (root); LS, *Lannea schweinfurthii* roots; ZMM, *Ziziphus mucronata* roots; CBR, *Crinum bulbispermum* (roots); TS, *Terminalia sericea* roots; trolox (positive control).

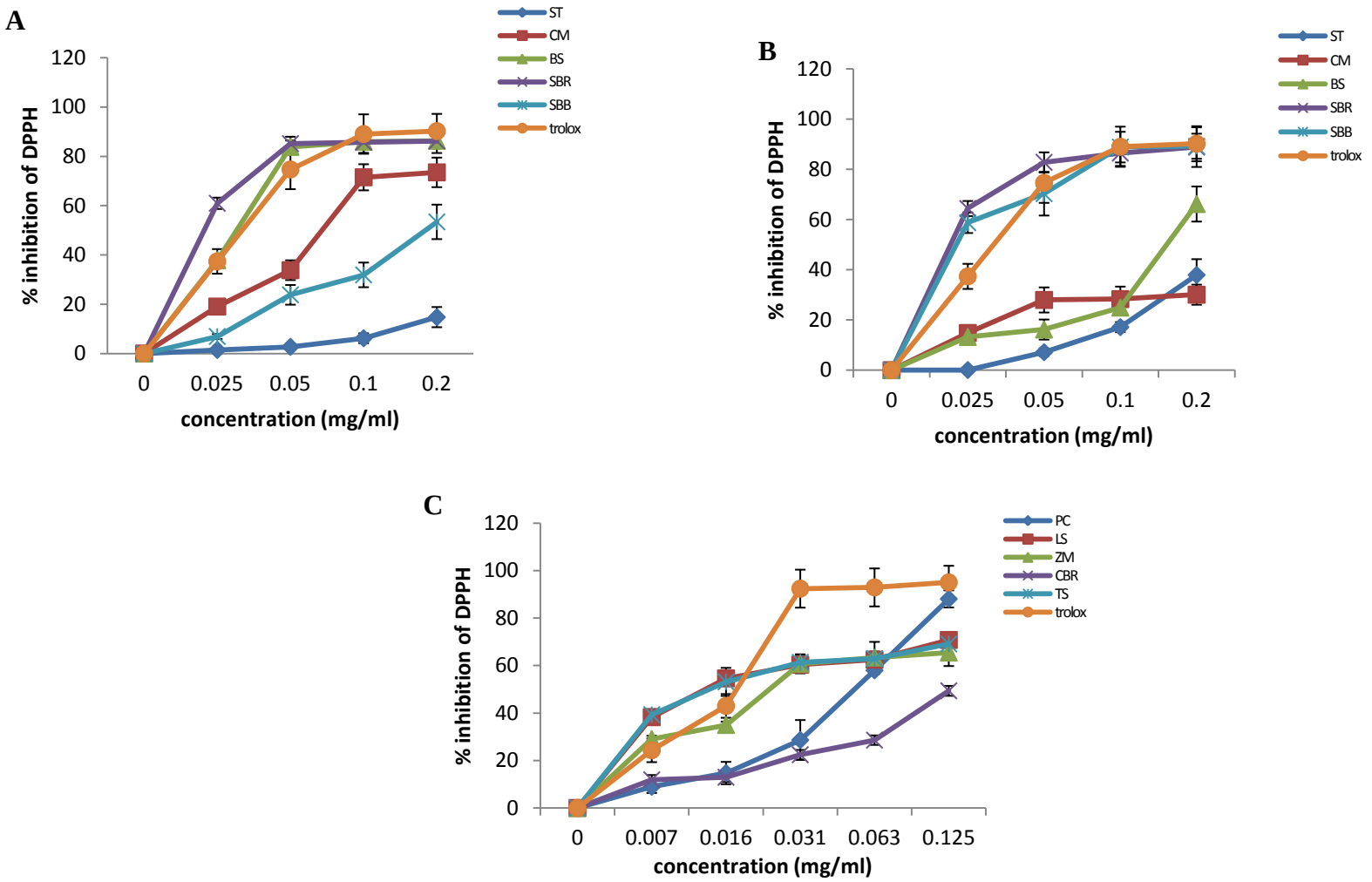


Figure 3.4 DPPH radical scavenging activity of (A) DCM: MeOH (1:1) extracts, (B) water extracts and (C) methanol extracts, of plants with good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *Schotia brachypetala* (bark); PC, *Piper capense* (root); LS, *Lannea schweinfurthii* roots; ZM, *Ziziphus mucronata* roots; CBR, *Crinum bulbispermum* (roots); TS, *Terminalia sericea* roots; trolox (positive control).

Table 3.2 Antioxidant activity of the plant extracts as represented by their IC_{50} values.

Species	Plant part analyzed	Extraction solvent	ABTS radical inhibition IC_{50} (mg/ml)	DPPH radical inhibition IC_{50} (mg/ml)
Salvia tiliifolia	Whole plant	DCM:MeOH (1:1)	*	*
		Water	1.5100 ± 0.230	*
Chamaecrista mimosoides	Root	DCM:MeOH (1:1)	0.3000 ± 0.050	0.7200 ± 0.030
		Water	*	*
Buddleja salviifolia	Whole plant	DCM:MeOH (1:1)	0.1400 ± 0.080	0.2300 ± 0.010
		Water	1.0000 ± 0.050	1.6000 ± 0.510
Schotia brachypetala	Root	DCM:MeOH (1:1)	$3.2600 \times 10^{-7} \pm 0.100 \times 10^{-9}$	0.0500 ± 0.020
		Water	$3.7000 \times 10^{-7} \pm 0.210 \times 10^{-9}$	0.0500 ± 0.020
	Bark	DCM:MeOH (1:1)	*	1.9000 ± 0.500
		Water	0.1500 ± 0.030	0.1300 ± 0.030
Adenia gummifera	Root	Methanol	*	*
		Ethyl acetate	*	*
Piper capense	Root	Methanol	0.0402 ± 0.003	0.0443 ± 0.010
		Ethyl acetate	*	*
Zanthoxylum davyi	Root	Methanol	0.0752 ± 0.021	*
		Ethyl acetate	*	*
Xysmalobium undulatum	Root	Methanol	*	*
		Ethyl acetate	*	*
Lannea schweinfurthii	Root	Methanol	0.0076 ± 0.001	0.0151 ± 0.004
		Ethyl acetate	*	*
Terminalia sericea	Root	Methanol	0.0071 ± 0.001	0.0147 ± 0.006
		Ethyl acetate	0.0746 ± 0.017	*
Ziziphus mucronata	Root	Methanol	0.0187 ± 0.020	0.0291 ± 0.051
		Ethyl acetate	*	*
Tabernaemontana elegans	Root	Methanol	*	*
		Ethyl acetate	*	*
Ficus capensis	Fruits	Methanol	*	*
		Ethyl acetate	*	*
Scadoxus puniceus	Bulb	Methanol	*	*
		Ethyl acetate	*	*
	Root	Methanol	*	*
		Ethyl acetate	*	*

Crinum bulbispermum	Bulb	Methanol	*	*
		Ethyl acetate	*	*
	Root	Methanol	0.0685 ± 0.041	*
		Ethyl acetate	*	*
Boophane disticha	Bulb	Methanol	*	*
		Ethyl acetate	*	*
	Root	Methanol	0.0913 ± 0.017	*
		Ethyl acetate	*	*
Tulbaghia violacea	Bulb	Methanol	*	*
		Ethyl acetate	*	*
	Root	Methanol	*	*
		Ethyl acetate	*	*
Control				
Trolox			0.1310 ± 0.0050	$0.96 \times 10^{-4} \pm 0.40 \times 10^{-6}$

* represents extracts with maximum inhibition below 50% at the highest concentration tested

Table 3.3 Total phenol and flavonoid contents of plant extracts with antioxidant activity (>50%) in both DPPH and ABTS assays.

Species	Plant part analyzed	Extraction solvent	Total Phenols[a]	Total Flavonoids[b]
Chamaecrista mimosoides	Root	DCM:MeOH (1:1)	141.53 ± 0.21	16.86 ± 0.35
		Water	64.16 ± 0.13	5.32 ± 0.38
Buddleja salviifolia	Whole plant	DCM:MeOH (1:1)	169.66 ± 0.33	23.95 ± 0.11
		Water	77.92 ± 0.91	12.11 ± 0.26
Schotia brachypetala	Root	DCM:MeOH (1:1)	303.91 ± 0.92	4.24 ± 0.23
		Water	291.80 ± 0.12	13.44 ± 0.08
	Bark	DCM:MeOH (1:1)	305.52 ± 0.21	10.97 ± 0.17
		Water	337.66 ± 0.12	17.71 ± 0.54
Crinum bulbispermum	Root	Methanol	202.38 ± 5.03	9.18 ± 0.50
Piper capense	Root	Methanol	237.60 ± 11.69	18.14 ± 1.97
Terminalia sericea	Root	Methanol	36.73 ± 2.07	73.05 ± 4.70
Lannea schweinfurthii	Root	Methanol	101.27 ± 1.60	13.58 ± 3.52
Ziziphus mucronata	Root	Methanol	73.86 ± 2.52	17.76 ± 2.29

[a]Expressed as mg tannic acid/g of extract

[b]Expressed as mg quercetin/g of extract

3.3 Isolation of compounds from *Boophane disticha*

Two compounds were isolated from the bulbs of *B. disticha*; 6-hydroxycrinamine from the methanol extract and cycloeucalenol from the ethyl acetate extract. In addition, two fractions which were active but could not be purified further because of their very low yield, were reserved and together with the isolated compounds, were screened for AChE inhibition. Studies on the cytotoxicity of the compounds and fractions were also carried out (section 3.4.1).

3.3.1 Structural elucidation of compound 1 (6 – hydroxycrinamine)

Compound 1 (6-hydroxycrinamine, $C_{17}H_{19}NO_5$), was isolated from the methanol extract of the bulbs of *B. disticha*. It was isolated as yellow crystals. The MS chromatogram (Figure 3.5; Appendix E: Figure 8), shows the (M + 1) ion at *m/z* 318 which corresponds to the reported molecular weight of 6-hydroxycrinamine (Viladomat et al., 1996). The fragment (M + 1 – 32) at *m/z* 286 corresponds to the loss of methanol, which confirms the presence of a methoxy group in the molecule. The molecular fragment ion peaks were determined as shown in Figure 3.5, (M + 1, $C_{17}H_{20}NO_5$) at *m/z* 318 with 300 (M + 1 – H_2O), 286 (– CH_4O), 268 (– CH_6O_2), 250 (– CH_8O_3), 240 (– $C_2H_6O_3$), 227 (– $C_4H_{11}O_2$) and 199 (– $C_5H_{11}O_3$).

The compound is very polar and was dissolved in deuterated methanol for NMR analysis (^{1}H, ^{13}C and 2D experiments). The ^{1}H and ^{13}C NMR (Appendix E: Figure 1 and 2), provided further evidence for the structure of the isolated compound. The NMR data was compared to that of the published data on 6-hydroxycrinamine (Viladomat et al., 1996), and the comparison is given in Table 3.4 (^{1}H NMR) and Table 3.5 (^{13}C NMR). The signals obtained from both NMR spectra were complex suggesting that compound 1 was a mixture of two epimers, epimer A (6α-hydroxycrinamine) (**3.1**) and epimer B (6β-hydroxycrinamine) (**3.2**) (Figure 3.6). From the data

obtained and comparison with literature data, the two epimers could be identified in the NMR spectra (Figure 3.6).

3.3.2 Structural elucidation of compound 3, 24-methylenecycloartan-3β-ol (cycloeucalenol)

Compound 3 (cycloeucalenol, $C_{30}H_{50}O$), was isolated from the ethyl acetate extracts of the bulbs of *B. disticha* as white crystals. The MS chromatogram (Figure 3.7; Appendix E: Figure 14), shows the (M + 1) ion at *m/z* 427, which corresponds to the reported molecular weight of cycloeucalenol (Deng et al., 2009). The molecular fragment ion peaks were determined (M + 1, $C_{30}H_{50}O$) at *m/z* 427 with 409 (M + 1 – H_2O), 343 (– C_6H_{12}), 327 (– $C_6H_{12}O$), 285 (– $C_9H_{18}O$), 219 (– $C_{14}H_{24}O$), 177 (– $C_{17}H_{30}O$) and 163 (– $C_{18}H_{32}O$).

The compound is non-polar and was dissolved in deuterated chloroform for NMR analysis (^1H, ^{13}C and 2D experiments). The NMR spectra of the compound after comparison with reported literature data confirmed it to be cycloeucalenol, as the major compound, together with its stereo-isomer. The two compounds co-chromatographed together and were thus not possible to separate with the available solvent systems. Similarly, Knapp and Nicholas (1970), isolated both compounds as a mixture and the authors were unable to separate the compounds. Cycloeucalenol and its stereo-isomer have the same skeletal structure (**3.3**). The only difference in their structures is in the side chain (**3.3a** and **3.3b**) (Figure 3.8). Cycloeucalenol has a double bond between position C-24 and C-30 while its stereo-isomer has a double bond between C-25 and C-27. The NMR data was compared to that of the published data on cycloeucalenol (Liu et al., 2011), and the comparison is given in Table 3.6 (^1H NMR) and Table 3.7 (^{13}C NMR). In addition, the data obtained for the ^{13}C-NMR spectrum of the stereo-isomer on the side-chain, also compares with data reported by Akihisa et al. (1997).

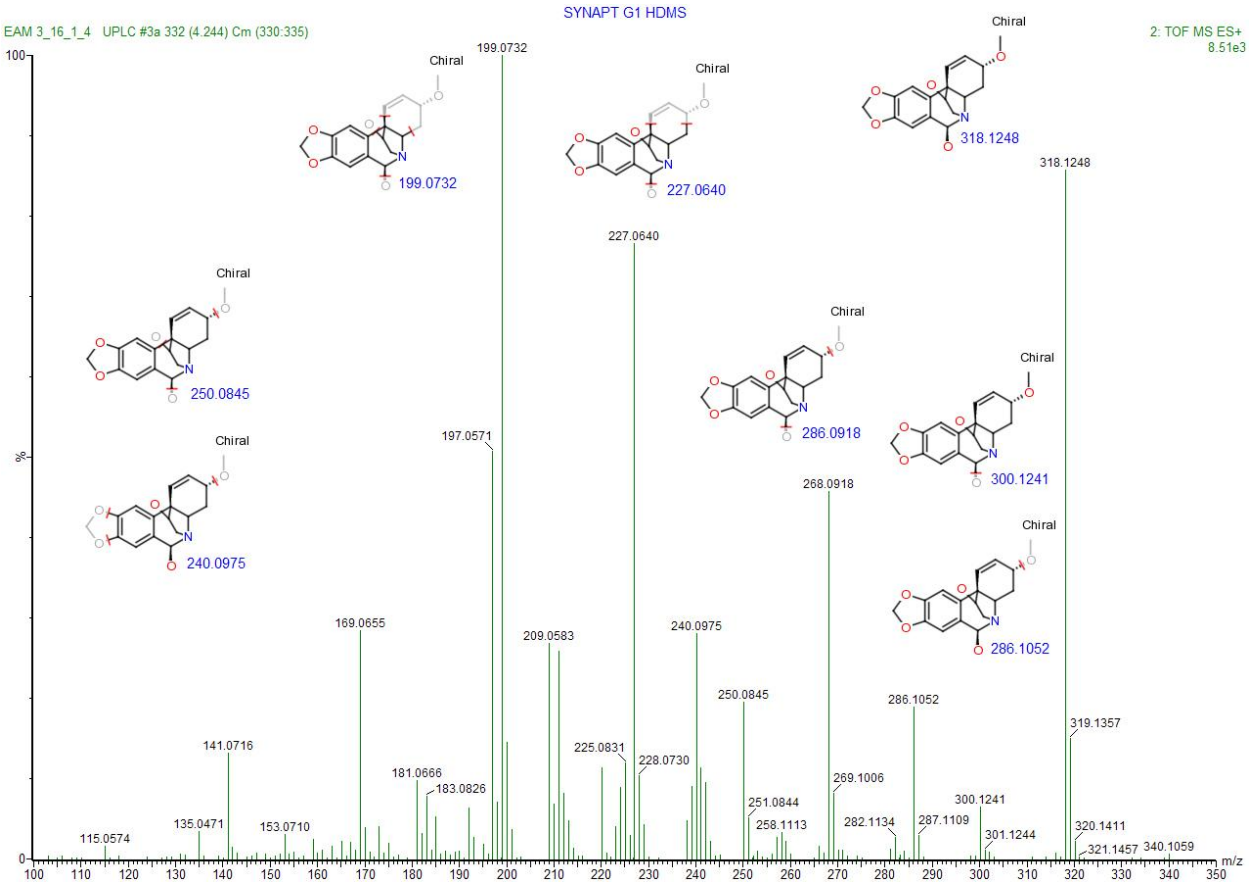
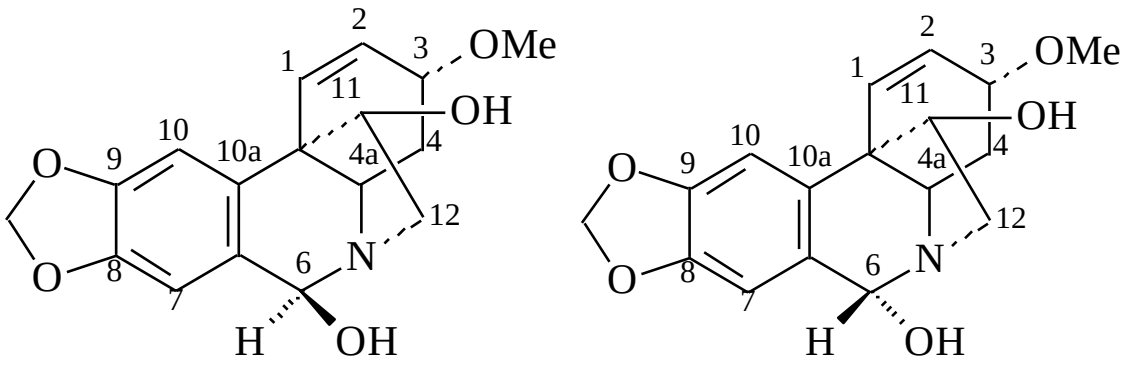


Figure 3.5 HRTOFMS (ESI^+) spectra for compound 1 (6-hydroxycrinamine).



Epimer A (6α-hydroxycrinamine) (**3.1**) Epimer B (6β-hydroxycrinamine) (**3.2**)

Figure 3.6 Structure of 6-hydroxycrinamine showing its two epimers.

Table 3.4 ^{1}H NMR data for 6-hydroxycrinamine in methanol-d_4 (CD_3OD) compared to literature.

H	**6α-hydroxycrinamine (Epimer A), δ_H (*J* in Hz)**	**Reference compound A*, δ_H (*J* in Hz)**	**6β-hydroxycrinamine (Epimer B), δ_H (*J* in Hz)**	**Reference compound B*, δ_H (*J* in Hz)**
1	6.22 *m*	6.23 *d* (10.5)	6.22 *m*	6.21 *d* (10.5)
2	6.22 *m*	6.19 *dd* (10.5, 2.0)	6.22 *m*	6.17 *dd* (10.5, 1.5)
3	4.02 *m*	4.02 *ddd* (9.0, 6.5, 2.0)	4.02 *m*	3.96 *ddd* (10.5, 6.0, 1.5)
4α	2.14 *m*	2.11 *ddd* (13.0, 12.5, 9.1)	2.26 *m*	2.26 *ddd* (13.5, 12.5, 10.0)
4β	2.07 *m*	2.08 *ddd* (12.5, 6.5, 5.0)	2.16 *m*	2.16 *ddd* (12.5, 6.0, 5.0)
4a	3.71 *brdd* (12.9, 5.0)	3.73 *ddd* (13.0, 5.0, 1.0)	3.42 *m*	3.41 *ddd* (13.0, 5.0, 1.0)
6	5.01 *s*	5.01 *s*	5.60 *s*	5.59 *s*
7	6.80 *s*	6.80 *s*	6.96 *s*	6.96 *s*
10	6.75 *s*	6.74 *s*	6.73 *s*	6.72 *s*
11	3.89 *m*	3.90 *ddd* (6.5, 3.0, 1.0)	3.89 *m*	3.87 *ddd* (7.0, 2.5, 1.0)
12 endo	3.36 *brd*	3.35 *dd* (14.0, 6.5)	4.19 *dd* (14.1, 6.5)	4.19 *dd* (14.0, 7.0)
12 exo	3.33 *dd* (14.1, 2.9)	3.30 *dd* (14.0, 3.0)	3.02 *brd*	3.01 *dd* (14.0, 2.5)
OCH_2O	5.90 *brd*	5.89 *d*-5.91 *d* (1.5)	5.90 *brd*	5.88 *d*-5.90 *d* (1.5)
3-OMe	3.39 *s*	3.37 *s*	3.39 *s*	3.38 *s*

*Literature data from Viladomat et al., 1996

Table 3.5 ^{13}C NMR data for 6-hydroxycrinamine in methanol-d_4 (CD_3OD) compared to literature.

Carbon atom	**6α-hydroxycrinamine (Epimer A), δ_C/ppm**	**Reference compound A*, δ_C/ppm**	**6β-hydroxycrinamine (Epimer B), δ_C/ppm**	**Reference compound B*, δ_C/ppm**
1	136.9	136.4	136.8	136.2
2	123.3	123.0	123.1	123.2
3	76.2	75.9	75.8	75.6
4	29.8	29.4	29.6	29.4
6	88.4	88.0	85.9	85.5
6a	127.5	127.3	127.5	128.8
7	109.7	109.5	108.5	108.3
8	147.0	146.5	146.8	146.7
9	148.1	147.8	147.9	147.5
10	103.1	102.8	103.0	102.7
10a	136.2	135.8	134.9	134.6
10b	51.1	50.4	50.7	50.8
11	78.4	78.1	79.3	79.0
12	58.1	57.7	52.1	51.8
-OCH_2O	102.9	101.1	101.3	101.1
3-OMe	56.2	55.9	56.2	55.9

*Literature data from Viladomat et al., 1996

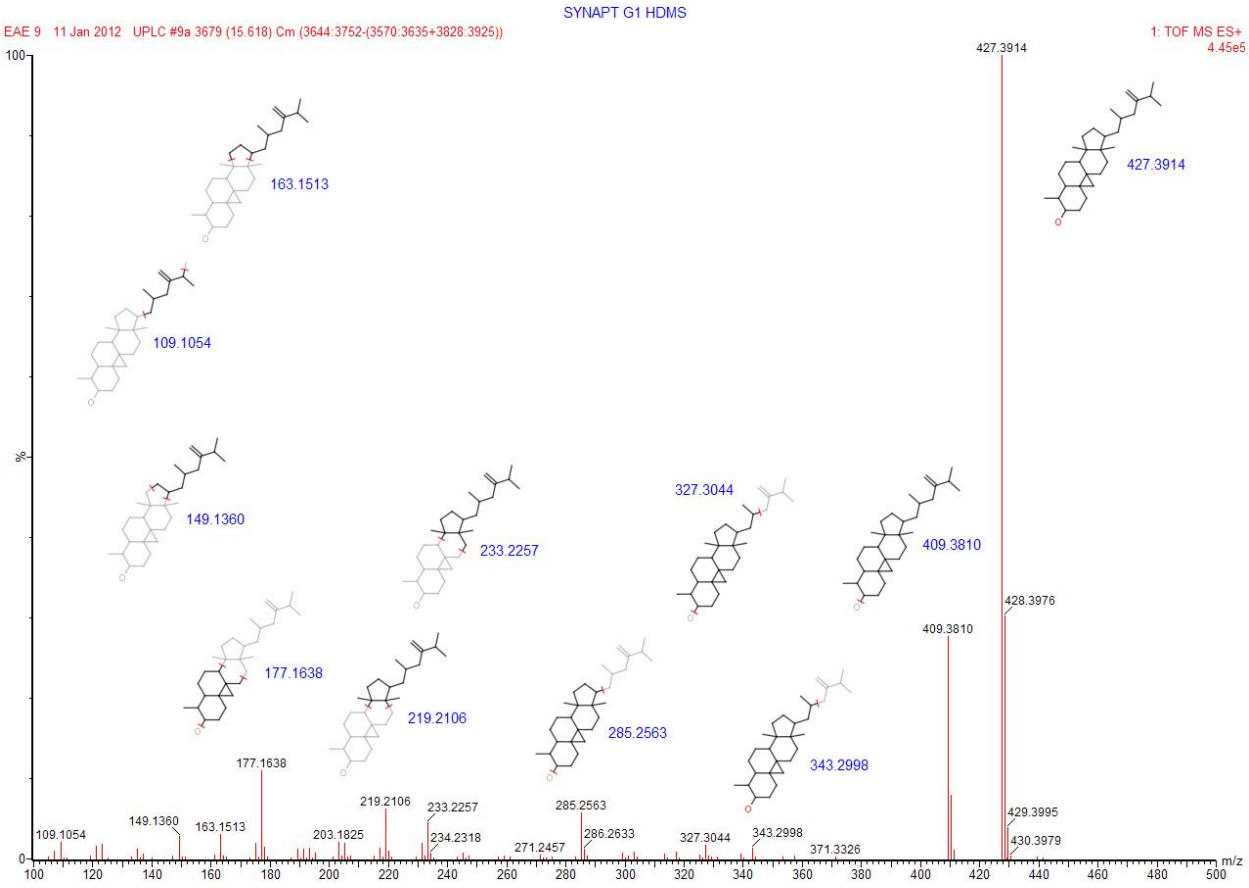
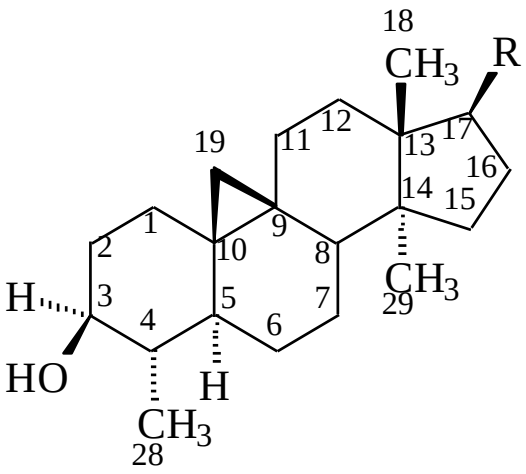
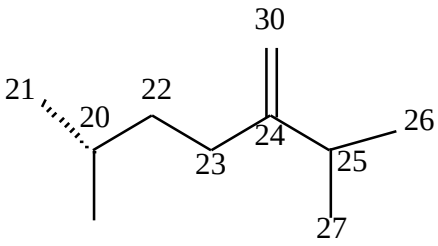


Figure 3.7 HRTOFMS (ESI^+) spectra for compound 3 (cycloeucalenol).

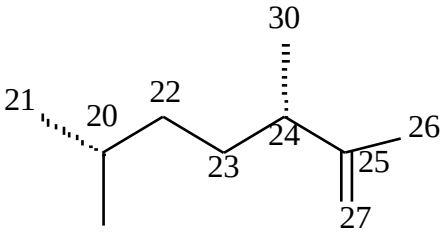


3.3

Side chain (R)



3.3a



3.3b

Figure 3.8 Structure of cycloeucalenol (3.3a) with its stereo-isomer (3.3b).

Table 3.6 ^{1}H NMR data for cycloeucalenol in chloroform-d_1 ($CDCl_3$) compared to literature.

H	**Reference compound*, δ_H (*J* in Hz)**	**(24,30)-cycloeucalenol (3.3a), δ_H (*J* in Hz)**	**Stereoisomer, (25,27)-cycloeucalenol (3.3b), δ_H (*J* in Hz)**	**Reference compound stereoisomer#, δ_H (*J* in Hz)**
3	3.22 *m*	3.19 *m*		
18	0.97 *s*	0.95 *s*		
19a	0.39 *d* (3.8)	0.37 *brs*		
19b	0.14 *d* (3.8)	0.12 *brs*		
21	0.91 *brs*	0.94 *brs*	0.86 *brs*	0.86 *d* (6.6)
24			2.22 *hextet* (7.0)	2.09 *hextet* (7.0)
26	1.03 *d* (6.8)	1.02 *d* (6.6)	1.64 *m*	1.64 *t* (1.4)
27	1.03 *d* (6.8)	1.00 *d* (6.6)	4.64 *brs*	4.67 *t* (1.4)
28	0.89 *s*	0.86 *s*	0.95 *brs*	0.94 *d* (7.1)
29	0.98 *d* (5.9)	0.97 *brs*	0.88 *s*	0.89 *s*
30a	4.72 *brs*	4.70 *brs*	1.00 *d* (6.6)	1.00 *d* (6.9)
30b	4.67 *brs*	4.64 *brs*		

* Literature data for cycloeucalenol from Liu et al., 2011.

Literature data for stereoisomer from Akihisa et al., 1997.

Table 3.7 ^{13}C NMR data for cycloeucalenol in chloroform-d_1 ($CDCl_3$) compared to literature.

Carbon atom	**Reference compound* δ_C/ppm**	**(24,30)-cycloeucalenol (3.3a), δ_C/ppm**	**Stereoisomer, (25,27)-cycloeucalenol (3.3b), δ_C/ppm**
1	30.8	31.0	31.0
2	34.8	35.0	35.0
3	76.6	76.8	76.8
4	44.6	44.8	44.8
5	43.3	43.6	43.6
6	24.7	24.9	24.9
7	28.1	28.3	28.3
8	46.9	47.1	47.1
9	23.5	23.8	23.8
10	29.5	29.8	29.8
11	25.2	25.4	25.4
12	35.3	35.6	35.6
13	45.3	45.5	45.6
14	48.9	49.1	49.1
15	32.9	33.1	33.1
16	27.0	27.2	27.2
17	52.2	52.4	52.4
18	17.8	18.0	18.0
19	27.2	27.5	27.5
20	36.1	36.3	36.4
21	18.3	18.6	18.6
22	35.0	35.2	34.0
23	31.3	31.5	31.7
24	156.9	157.2	41.8
25	33.8	34.2	150.5
26	22.0	22.2	18.9
27	21.9	22.1	109.6
28	14.4	14.6	14.6
29	19.1	19.4	19.4
30	105.9	106.2	22.1

* Literature data for cycloeucalenol from Liu et al., 2011.

3.3.3 Acetylcholinesterase inhibitory activity of isolated compound and fractions

In the bioassay guided purification of the compounds, AChE inhibitory activity of each fraction was assessed using the TLC method as described in section 2.3.2. The concentration of the isolated compounds and fractions that showed a 50% inhibition of enzyme activity (IC_{50}) was determined by a microtiter plate assay based on Ellman's method (Eldeen et al., 2005), as described in section 2.3.1.

From the TLC bioautographic assay, compound 1 or 6-hydroxycrinamine (EAM 3,16 1-4), compound 2 (EAE 1) and fractions EAM 17-21 21,22 and EAE 11 all showed activity (results not shown). No false positive reactions were seen (results not shown). However, compound 2 was unstable and degraded on storage before NMR analysis could be carried out. Compound 3 or cycloeucalenol (EAE 9), was not active for inhibition of AChE.

The compound and fractions which showed activity were further screened using the microtiter plate assay to determine their IC_{50} values for inhibition of AChE. The IC_{50} values obtained for inhibition of AChE by compound 1 and fractions EAM 17-21 21,22 and EAE 11 are provided in Table 3.8. The IC_{50} value of 6-hydroxycrinamine was expressed as a molar concentration since its structure and molecular weight could be determined while that of the fractions were expressed as mg/ml.

Table 3.8 *In vitro* AChE inhibitory activity of the isolated compound and fractions.

Compound/Fractions	**AChE inhbitory activity (IC_{50} values)**
6-hydroxycrinamine (compound 1)	0.445 ± 0.030 mM
EAM 17-21 21,22	0.067 ± 0.005 mg/ml
EAE 11	0.122 ± 0.013 mg/ml

3.4 Cytotoxicity studies

3.4.1 Cytotoxicity assessment and effect of isolated compounds and active fractions on Aβ-induced neurotoxicity

The effect of the isolated compounds and active fractions, on viability of SH-SY5Y cells are presented in Figure 3.9. Both the compounds and active fractions had a dose-dependent effect on viability. Results obtained from both cytotoxicity assays (MTT and neutral red uptake), were comparable. 6-hydroxycrinamine was the more toxic of the two compounds isolated with IC_{50} values of 61.7 µM and 54.5 µM for the neutral red and MTT assays, respectively (Table 3.9). EAM 17-21 21,22 was also the more toxic of the two active fractions with IC_{50} values of 36.9 µg/ml and 21.5 µg/ml for the neutral red and MTT assays, respectively.

The compounds and fractions were further tested at non-toxic doses to evaluate their possible protective effect against $A\beta_{25-35}$ induced cell death. None of the four samples showed any protective effect (results not shown).

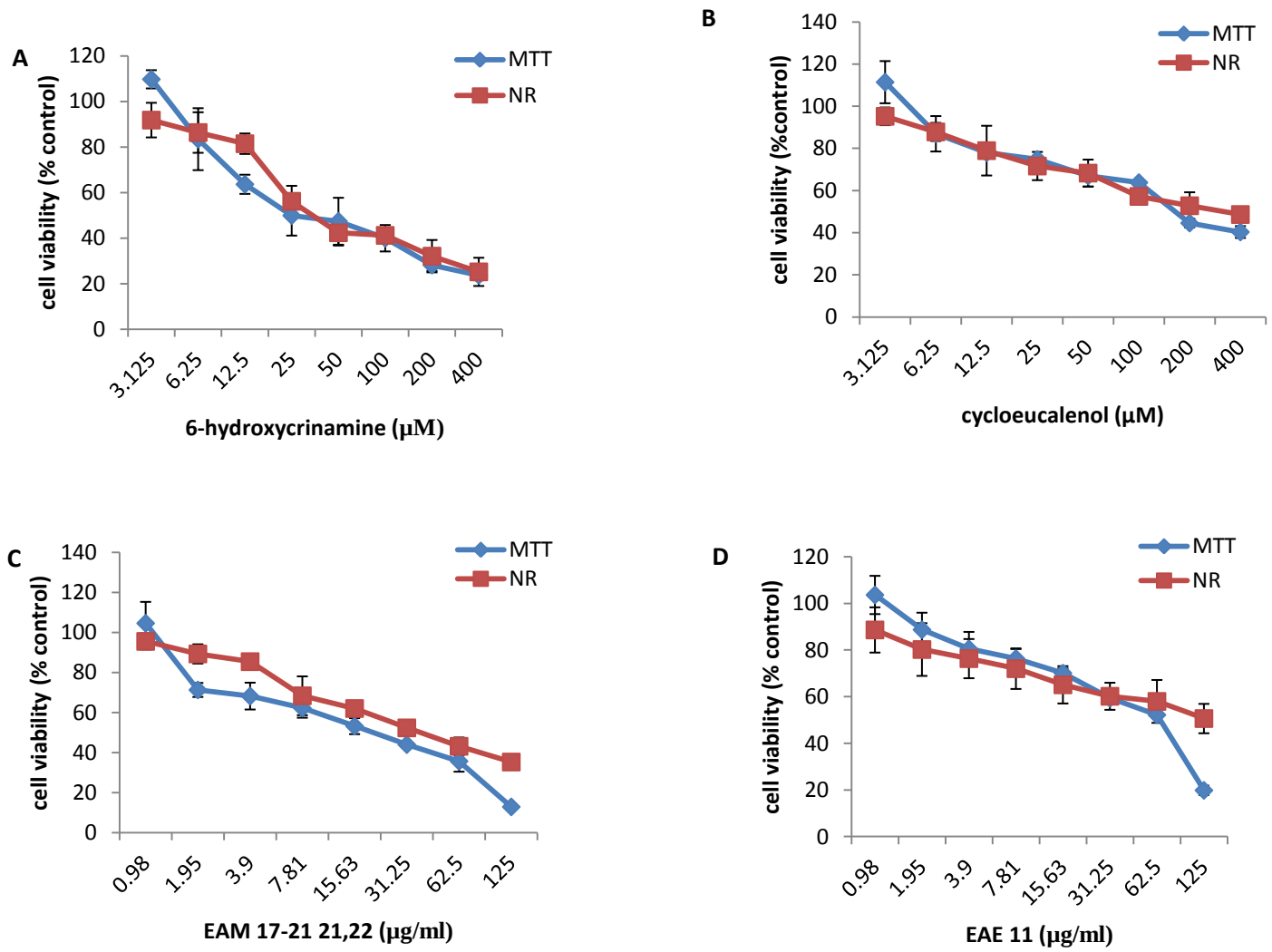


Figure 3.9 Effect of (A) 6-hydroxycrinamine, (B) cycloeucalenol, (C) EAM 17-21 21,22 and (D) EAE 11 on the viability of SH-SY5Y cell lines as measured by the MTT and neutral red uptake assays after 72 h of incubation.

Table 3.9 IC_{50} values of isolated compounds and fractions on SH-SY5Y cell lines.

Compound/Fraction	**Neutral Red assay (IC_{50} values)**	**MTT assay (IC_{50} values)**
6-hydroxycrinamine	61.7 ± 4.5 µM	54.5 ± 2.6 µM
cycloeucalenol	223.0 ± 6.4 µM	173.0 ± 5.1 µM
EAM 17-21 21,22	36.9 ± 3.5 µg/ml	21.5 ± 1.6 µg/ml
EAE 11	99.2 ± 5.8 µg/ml	83.7 ± 4.1 µg/ml

3.4.2 Cytotoxicity assessment and effect of several medicinal plants on Aβ-induced neurotoxicity

All the four plant extracts tested affected cell viability. The results, presented as percentage of cell growth compared to the untreated control for SH-SY5Y cells are presented in Figure 3.10. The cytotoxic effect of the four plant extracts, as represented by their IC_{50} values are shown in Table 3.10. The results obtained from both the neutral red and MTT assays were comparable. *C. bulbispermum* root extract was the most toxic, reducing cell viability by <40% at the highest concentration tested. Root extracts of *Z. mucronata* and *L. schweinfurthii* were the least toxic with IC_{50} values exceeding 100 µg/ml, the highest concentration tested.

Three concentrations that were not toxic, or presented low toxicity (from the results obtained in the cytotoxicity assays), were selected to evaluate their possible protective effect against cell death induced by $A\beta_{25-35}$. Treatment with $A\beta_{25-35}$ decreased cell viability to 16% of viable cells at the highest concentration (20 µM) tested (Fig. 3.11). Pretreatment with *Z. mucronata* and *T. sericea* roots showed a dose dependent inhibition of cell death caused by $A\beta_{25-35}$ (Fig. 3.12). Pre-treatment with *L. schweinfurthii* roots resulted in an optimum dose for inhibition of $A\beta_{25-35}$ induced cell death at 25 µg/ml, while still maintaining 80% viability (Fig. 3.12). The roots of *C. bulbispermum* at non-toxic dose still maintained >50% viability.

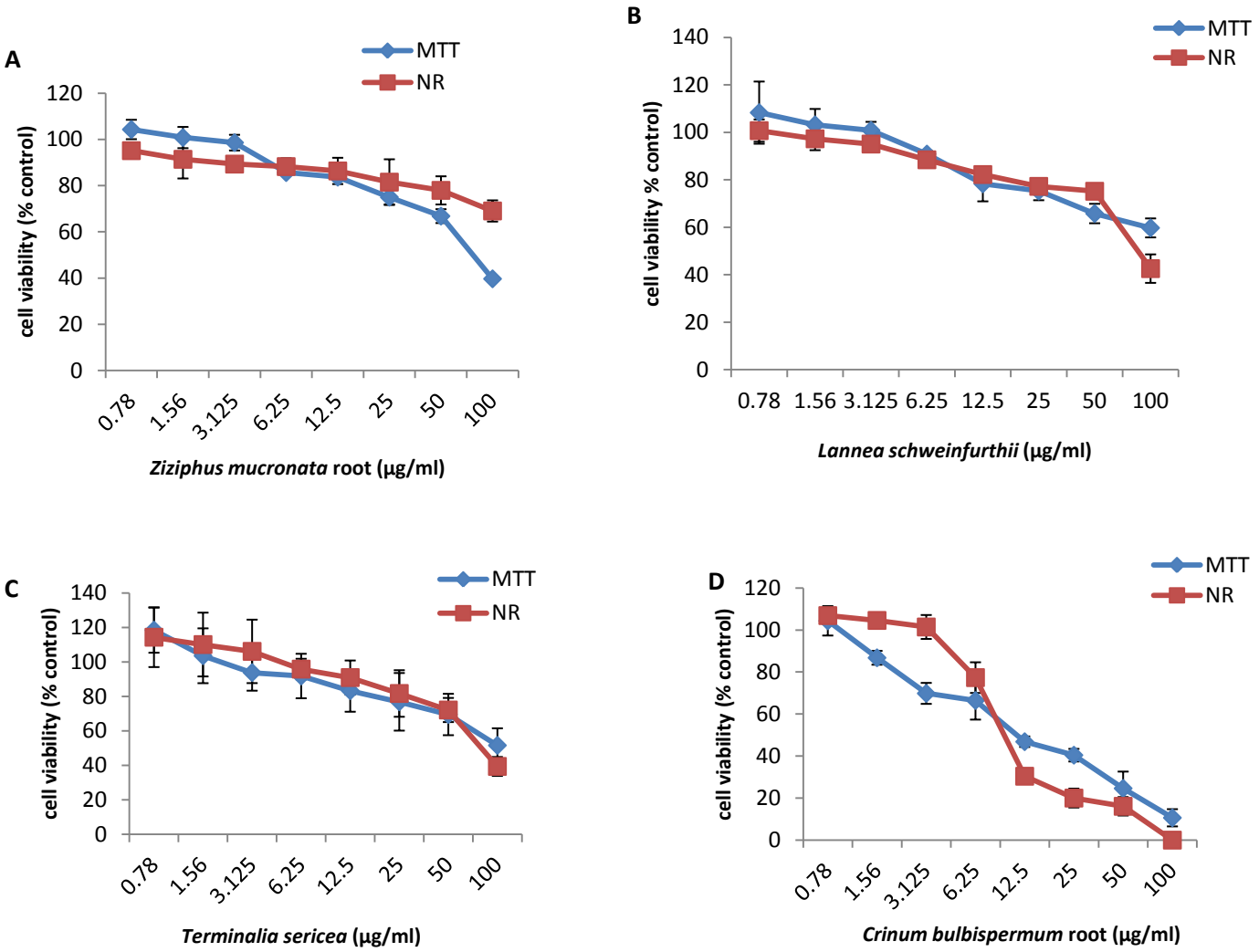


Figure 3.10 Effect of the methanol extract of the investigated plant extracts on viability of SH-SY5Y cell lines as measured by the MTT and neutral red uptake assays after 72 h of incubation (A) *Z. mucronata* roots; (B) *L. schweinfurthii* roots; (C) *T. sericea* roots; (D) *C. bulbispermum* roots.

Table 3.10 IC_{50} values of the methanol extracts of the investigated plants on SH-SY5Y cell lines.

Plant and part	**Neutral red assay IC_{50} (µg/ml)**	**MTT assay IC_{50} (µg/ml)**
Z. mucronata roots	>100	>100
L. schweinfurthii roots	>100	>100
T. sericea roots	100.72 ± 3.77	95.14 ± 2.45
C. bulbispermum roots	10.71 ± 0.26	12.53 ± 0.89

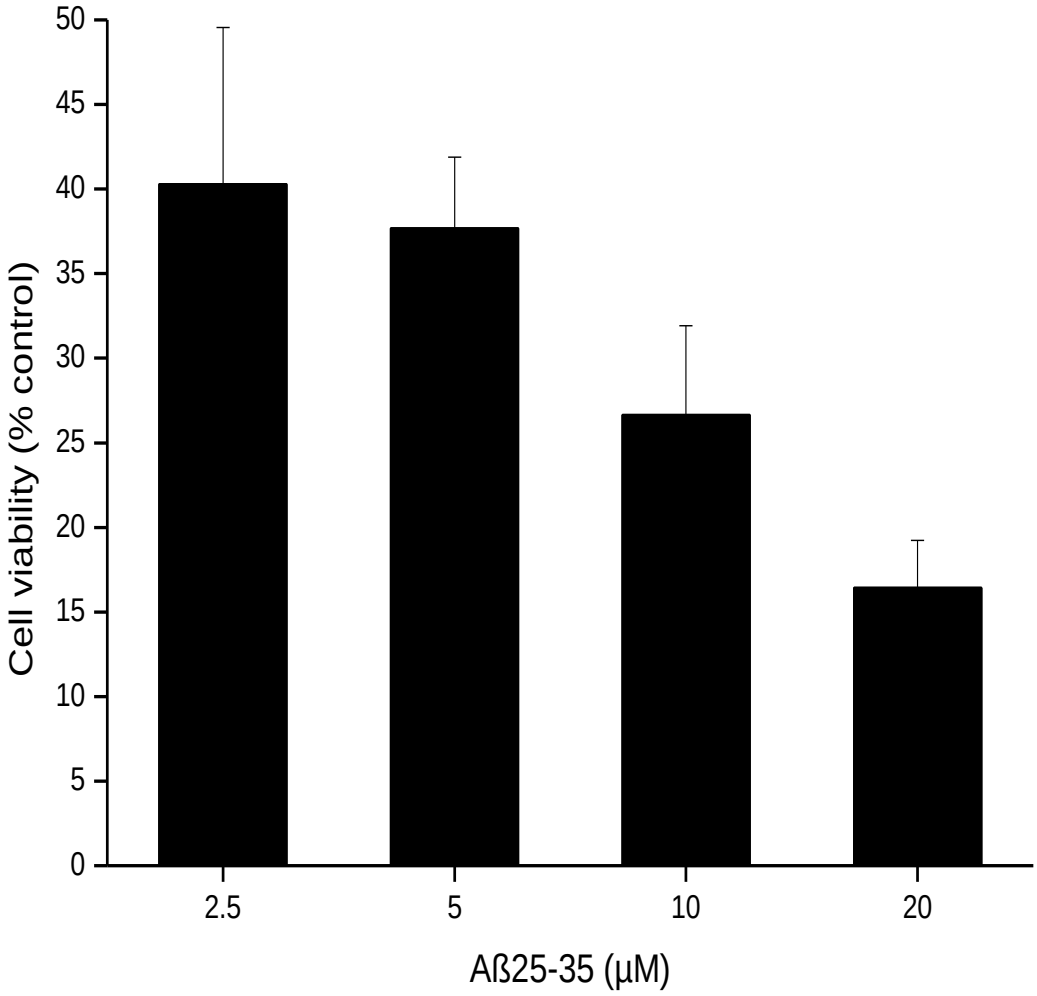


Figure 3.11 Effect of $A\beta_{25-35}$ on SH-SY5Y cell viability.

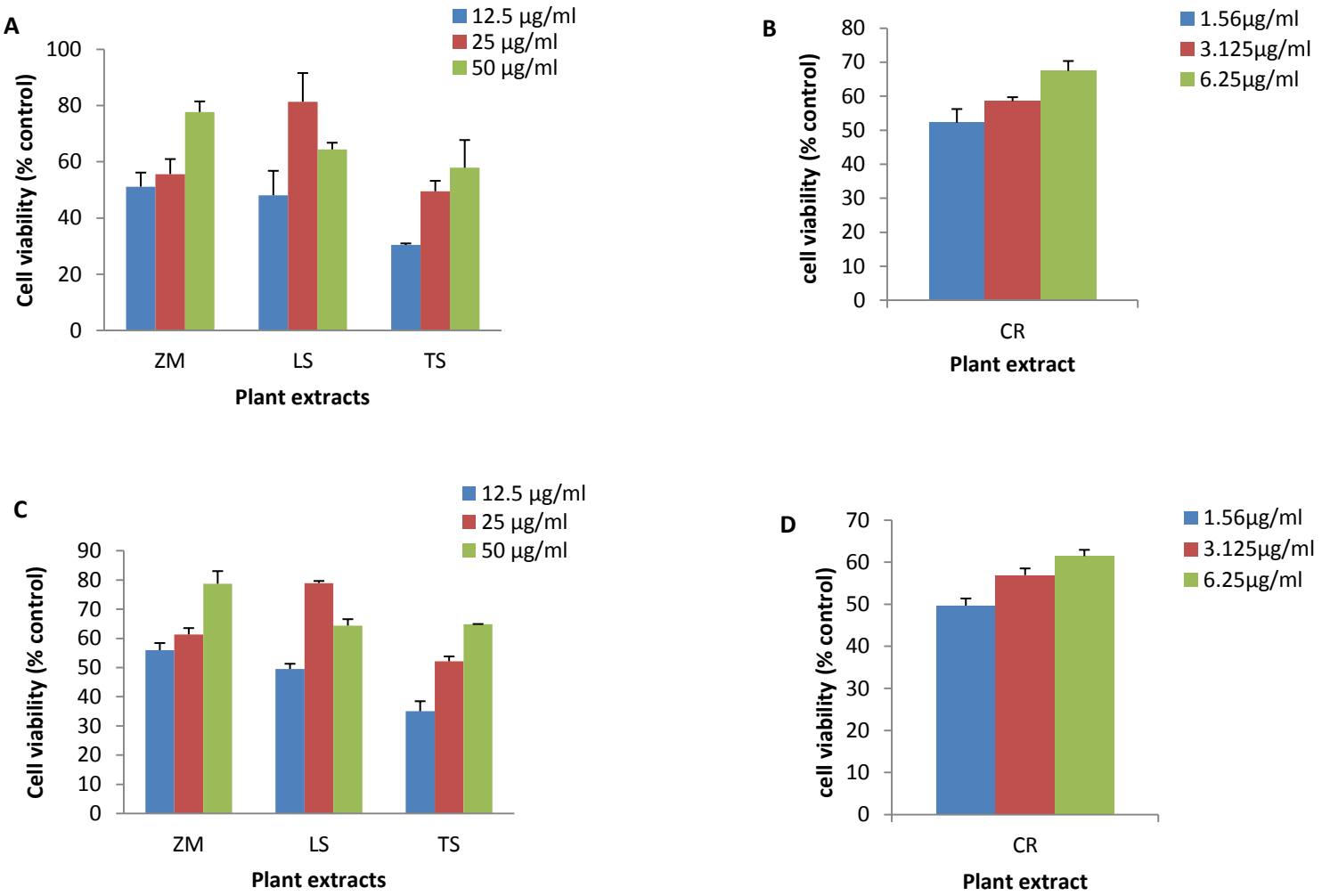


Figure 3.12 Effect of different plant extracts on Aβ25-35 induced toxicity. (A) and (B) Neutral red assay; (C) and (D) MTT assay. ZM, *Z. mucronata* root; LS, *L. schweinfurthii* root; TS, *T. sericea* root; CR, *C. bulbispermum* root.