

CHAPTER IV

RESULT AND DISCUSSION

4.1 GC-MS Results

The compounds identification was performed by analyzing the MS spectral pattern based on the GC-MS NIST08 database library and comparison with literature data. Most of the compounds identified have similarity index more than 90%. The GC-MS chromatogram is shown in Appendices, and the corresponding compounds with their retention times, peak's area, molecular formula, molecular weights (MW), and similarity index (%) in the tables (4.1; 4.2; 4.3; 4.5; 4.6; 4.7 and 4.8).

4.1.1 Chloroform Extract

The chromatogram of chloroform extract shown in Table 4.1 19 peaks taking into consideration only those compounds which have peak area higher than 1%. Six of those peaks were prominent which were identified as 9,12,15-Octadecatrienoic acid, ethyl ester ($C_{20}H_{34}O_2$) with retention time of 30.59 min and peak area of 42.52 %; Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) with retention time of 28.20 min and peak area of 9.55 %; Ethanol, 2-(9,12-octadecadienyloxy)- ($C_{20}H_{38}O_2$) with retention time of 30.47 min and peak area 5.36 %; Octadecatrienoic acid, ethyl ester ($C_{18}H_{36}O_2$) with retention time of 30.85 min and peak area 4.25 %; squalene ($C_{30}H_{50}$) with retention time of 37.76 min and peak 4.23 %; and 2-butanol, 1-mercapto- ($C_4H_{10}OS$) with retention time of 17.42 min and peak 3.68 %.

4.1.2 Hexane Extract

The hexane extract shown in Table 4.2. 18 peaks those have peak area higher than 1%. 6 of those peaks were prominent which were 9,12,15-Octadecatrienoic acid, methyl ester ($C_{19}H_{32}O_2$) with retention time of 29.71 min and peak area of 9.97 %; phytol ($C_{20}H_{40}O$) with retention time of 29.79 min and peak area of 7.35 %; Methyl-5,11,14,17-eicosatetraenoate ($C_{21}H_{34}O_2$) with retention time of 32.07 min and peak area 7.23 %; n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with retention time of 27.95 min and peak area 6.80 %; Cis,cis-7,10,13-Hexadecatrienal ($C_{16}H_{26}O$) with retention time of 30.57 min and peak area 5.38 %; and Tetratetracontane ($C_{44}H_{90}$) with retention time of 38.55 min and peak 4.99 %.

4.1.3 Methanol Extract

The methanol extract shown in Table 4.3. 8 peaks those have peak area higher than 1%. Three of those peaks were prominent which were β -D- Glucopyranoside, methyl ($C_7H_{14}O_6$) with retention time of 23.65 min and peak area of 68.63 %; Chloroethyl 2-hexyl ether ($C_8H_{17}ClO$) with retention time of 19.74 min and peak area of 7.55 %; N, 2-[3-Trimethylsilylpropoxy] ethyl amino ethane thiosulfuric acid ($C_{10}H_{25}NO_4S_2S$) with retention time of 17.56 min and peak area of 4.69 %.

4.1.4 Chloroform; Hexane (50 %; 50 %) Extract

Chloroform; Hexane (50 %; 50 %) extract shown in Table 4.4. 17 peaks those have peak area higher than 1%. 6 of those peaks were prominent which were n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with retention time of 27.97 min and peak area of 18.30 %; 9,12,15-Octadecatrienoic acid ($C_{20}H_{40}O$) with retention time of 30.45 min and peak area of

18.04%; 9,12,15-Octadecatrienoic acid, ethyl ester ($C_{20}H_{34}O_2$) with retention time of 30.57 min and peak area of 16.45 %; Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) with retention time of 28.20 min and peak area of 4.06 %; 2,4-pentadien-1-ol, 3-pentyl ($C_{10}H_{18}O$) with retention time of 31.02 min and peak area of 3.40 %; 2,4-pentadien-1-ol, 3-pentyl ($C_{20}H_{40}O_2$) with retention time of 30.85 min and peak area of 3.02 %.

4.1.5 Chloroform; Methanol (50 %; 50 %) Extract

Chloroform; Methanol (50 %; 50 %) extract shown in Table 4.5. 16 peaks those have peak area higher than 1%. 6 of those peaks were prominent β -D-Glucopyranoside, Methyl ($C_7H_{14}O_6$) with retention time of 22.63 min and peak area of 27.59 %; Vitamin E ($C_{29}H_{50}O_2$) with retention time of 41.00 min and peak area of 8.62 %; Phytol ($C_{20}H_{40}O$) with retention time of 29.77 min and peak area of 7.31 %; β -sitosterol ($C_{29}H_{50}O$) with retention time of 42.91 min and peak area of 5.72 %; 2,6,10,14,18,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl- ($C_{30}H_{50}$) with retention time of 37.75 min and peak area of 4.02 %; stigmasterol ($C_{29}H_{48}O$) with retention time of 42.35 min and peak area of 3.24 %.

4.1.6 Hexane; Methanol (50 %; 50 %) Extract

Hexane; Methanol (50 %; 50 %) extract shown in Table 4.6. 14 peaks those have peak area higher than 1%. 6 of those peaks were prominent β -D-Glucopyranoside, methyl ($C_7H_{14}O_6$) with retention time of 22.87 min and peak area of 27.46 %; phytol ($C_{20}H_{40}O$) with retention time of 29.78 min and peak area of 10.32 %; Chloroethyl 2-hexyl ether ($C_8H_{17}ClO$) with retention time of 19.63 min and peak area of 3.85 %; 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester ($C_{12}H_{23}NO_2$) with retention time

of 34.13 min and peak area of 2.86 %; N,2-[3-Trimethylsilylpropoxy]ethyl amino ethane thiosulfuric acid ($C_{10}H_{25}NO_4S_2SI$) with retention time of 17.47 min and peak area of 2.82 %; 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol ($C_{20}H_{40}O$) with retention time of 26.50 min and peak area of 2.09 %.

4.1.7 The mixture of Chloroform; hexane; methanol (33.33 %; 33.33 %; 33.33 %)

Extract

Chloroform; hexane; methanol (33.33 %; 33.33 %; 33.33 %) extract shown in Table 4.7. 18 peaks those have peak area higher than 1%. 6 of those peaks were prominent 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol ($C_{20}H_{40}O$) with retention time of 25.85 min and peak area of 15.48 %; Vitamin E ($C_{29}H_{50}O_2$) with retention time of 41.01 min and peak area of 12.91 %; β Sitosterol ($C_{29}H_{50}O$) with retention time of 42.93 min and peak area of 11.13 %; Squalene ($C_{30}H_{50}$) with retention time of 37.76 min and peak area of 7.84 %; Stigmasterol ($C_{29}H_{48}O$) with retention time of 42.36 min and peak area of 7.08 %; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester ($C_{19}H_{38}O_4$) with retention time of 34.84 min and peak area of 4.58 %.

4.1.8 Summary of The Compounds Detected:

In the GC-MS analyses of *Clinacanthus nutans* extracts, the identified compounds in the different extracts were tabulated in Table 4.8. The results showed that some compounds were identified in all extracts as phytol (acyclic diterpene alcohol) and its derivative 3,7,11,15-Tetramethyl-2-Hexadecan-1-ol. Some compounds were extracted with specific solvent. For example, Stigmasterol and β -Sitosterol were extracted with hexane and chloroform.

The mixture of the three solvents extracted 1-Dodecanol, 3,7,11-Trimethyl, Docosane, Trifluoroacetoxy hexadecane, dl-tocopherol, Gamma-sitosterol and Oxirane (Ethylene oxide). Most of these compounds have been previously reported in this plant (Dampawan et al., 1977; Sakdarat et al., 2009). The compounds in the solvent mixture extract, including Oxirane, Docosane, Trifluoroacetoxy hexadecane, dl-tocopherol and Gamma-sitosterol were assumed to be effective in inducing anticancer activity since they were only identified in this most active extract.

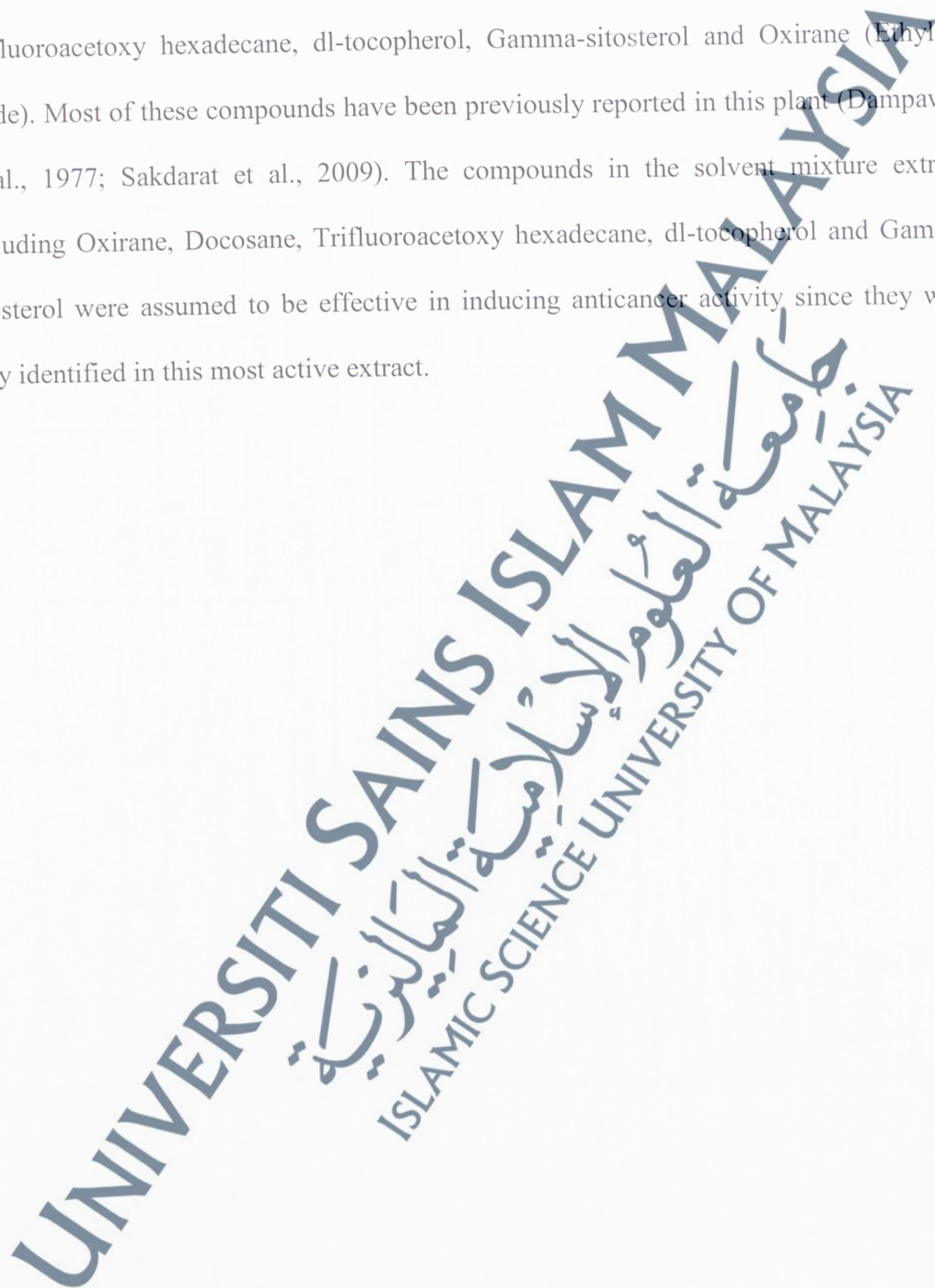


Table 4.1: Compounds detected in *Clinacanthus nutans* from Chloroform extract

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight	Similarity index (%)
2	9.67	1.21	Phenol	108-95-2	C ₆ H ₆ O	94	91
5	14.57	1.07	2-Hexen-1-ol	928-94-9	C ₆ H ₁₂ O	100	81
6	17.42	3.68	2-butanone, 1-mercapto-	25807	C ₄ H ₁₀ OS	106	70
12	25.66	1.23	cis-Z-6-Bisabolene epoxide	0-00-0	C ₁₅ H ₂₄ O	220	82
13	25.85	1.34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	92
24	28.10	1.08	Ethyl 9-hexadecenoate	54546-22-4	C ₁₈ H ₃₄ O ₂	282	91
25	28.20	9.55	Hexadecanoic acid, ethyl ester	628-97-7	C ₁₈ H ₃₆ O ₂	284	95
29	29.70	1.24	9,12,15-Octadecatrienoic acid, methyl ester	301-00-8	C ₁₉ H ₃₂ O ₂	292	96
32	30.47	5.36	Ethanol, 2-(9, 12- octadecadienylloxy)-	17367-08-7	C ₂₀ H ₃₈ O ₂	310	92
33	30.59	42.52	9,12,15-Octadecatrienoic acid, ethyl ester	1191-41-9	C ₂₀ H ₃₄ O ₂	306	97
34	30.85	4.25	Octadecatrienoic acid, ethyl ester	141-61-5	C ₁₈ H ₃₆ O ₂	284	93
48	37.76	4.23	Squalene	7683-64-9	C ₃₀ H ₅₀	410	97
50	38.55	1.10	Tettrariacontane	14167-69-0	C ₃₄ H ₇₀	478	96
57	40.42	2.09	Tettrariacontane	7098-22-8	C ₄₄ H ₉₀	618	96
58	41.00	1.28	Vitamin E	59-02-9	C ₂₉ H ₅₀ O ₂	430	90
62	42.17	1.28	Tettrariacontane	14167-59-0	C ₃₄ H ₇₀	478	90
63	42.36	1.86	Stigmasterol	83-48-1	C ₂₈ H ₄₈ O	412	94
66	42.92	3.23	β Sitosterol	83-46-5	C ₂₉ H ₅₀ O	414	95
69	43.65	1.13	Octadecanal	638-66-4	C ₁₈ H ₃₆ O	268	93

Table 4.2: Compounds detected in *Clinacanthus nutans* from Hexane extract

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index (%)
40	27.25	2.01	Hexadecanoic acid, methyl ester	112-39-0	C ₁₇ H ₃₄ O ₂	270	96
42	27.95	6.80	n-Hexadecanoic acid	57-10-3	C ₁₆ H ₃₂ O ₂	256	94
46	29.61	3.54	9,12 -Octadecatrienoic acid, methyl ester	2462-85-3	C ₁₉ H ₃₄ O ₂	294	95
47	29.71	9.97	9,12,15-Octadecatrienoic acid, methyl ester	301-00-8	C ₁₉ H ₃₂ O ₂	292	95
48	29.79	7.35	pinolol	150-86-7	C ₂₀ H ₄₀ O	292	96
51	30.57	5.38	Cis, cis-7,10,13-Hexadecatrienal	56797-43-4	C ₁₆ H ₂₆ O	234	91
52	31.01	4.10	2,4-pentadiene-1-ol, 3-pentyl-	0-00-0	C ₁₀ H ₁₈ O	154	85
57	32.14	2.48	Trans-13-octadecenoic acid	693-71.0	C ₁₈ H ₃₄ O ₂	282	87
59	32.49	2.04	Neoclovene-1(1), dihydro-	0-00-0	C ₁₅ H ₂₆	206	83
60	32.70	7.23	Methyl 5,11,14,17-tetrasatetraenoate	59149-01-8	C ₂₁ H ₃₄ O ₂	318	80
66	33.21	1.01	Trichloroacetic acid, trifluoro-2-ynyl ester	0-00-0	C ₁₅ H ₂₃ Cl ₃ O ₂	340	79
67	33.65	1.51	7-tetradecenal	65128-96-3	C ₁₄ H ₂₆ O	210	86
69	34.97	1.81	1,2-Benzenedicarboxylic acid, diisooctyl ester	27554-26-3	C ₃₀ H ₅₀ O ₄	278	96
77	37.76	2.82	squalene	7683-64-9	C ₃₀ H ₅₀	410	96
78	38.55	4.99	Tetratetracontane	7098-22-8	C ₄₄ H ₉₀	618	96
96	42.36	1.11	Stigmasterol	83-48-7	C ₂₉ H ₄₈ O	412	92
98	42.92	1.85	β Sitosterol	83-46-5	C ₃₀ H ₅₀ O	414	94
102	43.99	3.87	d-Norandrosterane (5.α.,14α)	6-00-0	C ₁₈ H ₃₀	246	80

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Table 4.3: Compounds detected in *Clinacanthus nutans* from Methanol extract

peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index (%)
10	11.90	1.09	S-Methyl methanethiosulphonate	2949-92-0	C ₂ H ₆ O ₂ S ₂	126	97
24	17.56	4.69	N,2-[3-Trimethylsilylpropoxyl]ethyl aminoethanethiosulfuric acid	0-00-0	C ₁₀ H ₂₅ NO ₄ S ₂ S	315	70
29	19.74	7.55	Chloroethyl 2-hexyl ether	0-00-0	C ₈ H ₁₇ ClO	164	76
30	20.01	1.68	Benzoofuran-2-carboxaldehyde	4265-16-1	C ₉ H ₆ O ₂	146	80
31	20.21	1.80	Benzoic acid 3-hydroxy-, methyl ester	19438-10-9	C ₈ H ₈ O ₃	152	78
33	21.07	1.03	β-D- Glucopyranose,1,6-anhydro	498-70-7	C ₆ H ₁₀ O ₅	162	94
37	23.65	68.63	β-D- Glucopyranoside, methyl	709-50-2	C ₇ H ₁₄ O ₆	194	97
48	29.16	1.37	n-Hexadecanoic acid	57-10-3	C ₁₆ H ₃₂ O ₂	256	81

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Table 4.4: Compounds detected in *Clinacanthus nutans* from Chloroform; Hexane (50 % - 50%) extract

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index (%)
45	25.85	1.24	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	93
60	27.97	18.30	n-Hexadecanoic acid	57-10-3	C ₁₆ H ₃₂ O ₂	256	95
61	28.108	1.16	Ethyl 9-hexadecenoate	54546-22-4	C ₁₈ H ₃₄ O ₂	282	90
62	28.20	4.06	Hexadecanoic acid, ethyl ester	628-97-7	C ₁₈ H ₃₆ O ₂	284	95
71	29.79	1.23	Pyhtol	150-86-7	C ₁₆ H ₃₂ O ₂	256	95
73	30.45	18.04	9,12,15-Octadecatrienoic acid	463-40-1	C ₂₀ H ₄₀ O	296	95
74	30.57	16.45	9,12,15-Octadecatrienoic acid, ethyl ester	1191-41-9	C ₂₀ H ₃₄ O ₂	306	97
75	30.85	3.02	Octadecanoic acid, ethyl ester	111-61-5	C ₂₀ H ₄₀ O ₂	312	92
76	31.025	3.40	2,4-pentadien-1-ol, 3-pentyl	0-00-0	C ₁₀ H ₁₈ O	154	85
78	32.150	1.80	Trans-17-Octadecenoic acid	693-71-0	C ₁₈ H ₃₄ O ₂	282	87
81	33.44	1.80	Tricyclo[20.8.0.0 ^(7,16)]triacetane-1(22),7(16)-diepoxy	0-00-0	C ₃₀ H ₅₂ O ₂	444	78
84	34.39	2.00	7-tetradecenal	65128-96-3	C ₁₄ H ₂₆ O	210	84
94	37.76	1.09	Squalene	7683-64-9	C ₃₀ H ₅₀	410	97
95	38.55	1.92	Tetratetracontane	7098-22-8	C ₄₄ H ₉₀	618	96
110	42.358	1.01	Stigmasterol	83-48-7	C ₂₉ H ₄₈ O	412	96
112	42.92	1.45	β Sitosterol	83-46-5	C ₂₉ H ₅₀ O	414	93
115	43.65	1.12	Octadecanal	638-66-4	C ₁₈ H ₃₆ O	268	93

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Table 4.5: Compounds detected in *Clinacanthus nutans* from Chloroform; Methanol (50 % - 50%) extract

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index (%)
8	11.88	1.78	S-Methyl methanethiosulphonate	2949-92-0	C ₂ H ₆ O ₂ S ₂	126	96
16	17.408	1.84	2-Butanol, 1-mercapto	25807-94-7	C ₄ H ₁₀ OS	106	70
17	18.217	1.24	Ethyl methyl sulphone	594-43-4	C ₃ H ₆ O ₂ S	108	71
20	19.55	2.65	Chloroethyl 2-hexyl ether	0-00-0	C ₈ H ₁₇ ClO	164	79
28	22.63	27.59	β-D-Glucopyranoside, Methyl	709-50-2	C₇H₁₄O₆	194	95
41	26.50	1.80	5,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	94
42	27.25	1.39	Pentadecanoic acid, 14-methyl-, methyl ester	5129-60-2	C ₁₇ H ₃₄ O ₂	270	95
51	29.77	7.31	Phytol	150-86-7	C₂₀H₄₀O	296	96
61	34.12	2.53	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0-00-0	C ₁₂ H ₂₃ NO ₂	213	89
67	34.83	1.70	Octadecanoic acid, 2,3-dihydroxypropyl ester	123-94-4	C ₂₁ H ₄₂ O ₄	358	90
71	36.900	1.07	Methyl (Z)-5,15,14,17-tetracosatetraenoate	59149-01-8	C ₂₁ H ₃₄ O ₂	318	91
73	37.75	4.02	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	111-02-4	C ₃₀ H ₅₀	410	96
79	41.00	8.62	Vitamin E	59-02-9	C ₂₉ H ₅₀ O ₂	430	97
80	42.15	1.30	5-Cholestene-3-ol, 24-methyl-	43-142-42	C ₂₈ H ₄₈ O	400	89
81	42.35	3.24	Stigmasterol	83-48-7	C ₂₈ H ₄₈ O	412	96
82	42.91	5.72	β - sitosterol	83-48-5	C ₂₉ H ₅₀ O	414	93

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Table 4.6: Compounds detected in *Clinacanthus nutans* from Methanol; Hexane (50 % - 50%) extract

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index(%)
12	11.89	1.85	S-Methyl methanethiosulphonate	2949-92-0	C ₂ H ₆ O ₂ S ₂	126	96
23	17.47	2.82	N-2-[3-Trimethylsilylpropoxy]ethyl aminoethanethiosulfuric acid	0-00-0	C ₁₀ H ₂₅ NO ₄ S ₂ SI	315	70
25	18.25	1.00	Ethyl methyl sulphone	594-43-4	C ₃ H ₈ O ₂ S	108	71
28	19.63	3.85	Chloroethyl 2-hexyl ether	0-00-0	C ₈ H ₁₇ ClO	164	78
29	20.01	1.53	Benzofuran-2-carboxaldehyde	4265-16-1	C ₉ H ₆ O ₂	146	75
34	22.87	27.46	β-D-Glucopyranoside, methyl	709-50-2	C ₇ H ₁₄ O ₆	194	96
50	26.50	2.09	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	95
58	29.70	1.47	9,12-Octadecadienoic acid, methyl ester	301-00-8	C ₁₉ H ₃₂ O ₂	292	95
59	29.78	10.32	Phytol	150-86-7	C ₂₀ H ₄₀ O	296	96
70	34.13	2.86	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0-00-0	C ₁₂ H ₂₃ NO ₂	213	90
74	34.83	1.80	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23470-00-0	C ₁₉ H ₃₈ O ₄	358	95
76	36.80	1.11	9,12-Octadecadienoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3443-82-1	C ₂₁ H ₃₈ O ₄	354	91
77	36.90	1.82	Methyl-5,11,14,17-eicosatetraenoate	59149-01-8	C ₂₁ H ₃₄ O ₂	318	92
79	37.75	1.00	Squalene	7683-64-9	C ₃₀ H ₅₀	410	96

❖ Bold color is refer to the compounds have peaks area higher than 3 %

Table 4.7: Compounds detected in *Clinacanthus nutans* from Chloroform; Hexane; Methanol (33.33 %; 33.33 %; 33.33 %) extract

Peak number	Retention time (min)	Peak's area (%)	Compound	CAS	Molecular formula	Molecular weight (MW)	Similarity index (%)
21	25.85	15.48	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	92
23	26.21	2.01 %	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	95
24	26.50	3.77 %	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	102608-53-7	C ₂₀ H ₄₀ O	296	95
33	29.77	2.98 %	Phytol	150-86-7	C ₂₀ H ₄₀ O	296	96
43	32.22	1.40 %	15-Hydroxypentadecanoic acid	4617-33-8	C ₁₃ H ₃₀ O ₃	258	81
54	34.12	1.15 %	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0-00-0	C ₁₂ H ₂₃ NO ₂	213	89
56	34.30	1.36 %	9,12-Octadecadienyl chloride	7459-33-8	C ₁₈ H ₃₁ ClO	298	89
57	34.40	1.33 %	Methyl-5,11,14,17-tetraoctatetraenoate	59149-01-8	C ₂₁ H ₃₄ O ₂	318	90
60	34.84	4.58 %	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23470-00-0	C ₁₉ H ₃₈ O ₄	330	94
72	36.80	3.55 %	9,12-Octadecadienoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3443-82-1	C ₂₁ H ₃₈ O ₄	354	92
73	36.90	3.80 %	Methyl-5,11,14,17-tetraoctatetraenoate	59149-01-8	C ₂₁ H ₃₄ O ₂	318	92
74	37.05	1.96 %	Octadecanoic acid, 2,3-dihydroxypropyl ester	123-94-4	C ₂₁ H ₄₂ O ₄	358	89
77	37.76	7.84 %	Squalene	7683-64-9	C ₃₀ H ₅₀	410	98
86	40.42	1.17 %	Tetratetracontane	7098-22-8	C ₄₄ H ₉₀	618	96
88	41.01	12.91 %	Vitamin E	10191-41-0	C ₂₉ H ₅₀ O ₂	430	95
91	42.15	3.00 %	Campesterol	474-62-4	C ₂₈ H ₄₈ O	400	92
92	42.36	7.08 %	Stigmasterol	83-48-7	C ₂₉ H ₄₈ O	412	96
95	42.93	11.13 %	β Sitosterol	83-47-6	C ₂₉ H ₅₀ O	414	92

❖ Bold color is refer to the compounds have peaks area higher than 3 %

No	Compound	Compounds availability, similarity index and retention time in <i>Clinacanthus nutans</i> extracts (SI / RT)							
		Chloroforms	Hexane	Methanol	Chloroform /Hexane	Chloroform/ Methanol	Methanol/ Hexane	Mixture of three solvents	
1	3-Hexenal	-	-	-	+	-	-	-	-
2	3-Hexen-1-ol	-	-	-	+	-	-	-	-
3	Butyrolactone	-	-	+	-	-	+	-	-
4	1-Octen-3-ol	+	+	-	+	-	-	91 / 7.99	-
5	Carbolic Acid	+	+	-	-	-	-	-	-
6	Glycerin	-	-	-	-	-	+	-	-
7	3-Octanol	-	-	-	+	-	-	93 / 9.70	-
8	2-Hydroxy-Gamma-Butyrolactone	-	-	+	-	-	+	-	-
9	Benzyl Alcohol	-	-	-	+	-	-	-	-
10	S-Methyl Methanethiosulphonate	+	-	+	-	+	+	-	-
11	Undecane	-	-	+	-	+	+	-	-
12	Phenylethyl Alcohol	-	-	-	-	-	-	-	-
13	4H-Pyran-4-One,2,3-Dihydro-3,5-Dihydroxy-6-Methyl	-	-	+	-	+	+	-	-
14	Benzoic Acid	-	+	-	+	-	-	-	-

30	Pentadecanoic Acid, 14-Methyl-,Methyl Ester	+	94 / 27.25	-	+	94 / 27.25	+	95 / 27.25	-	+	93 / 27.25
31	Eicosanic Acid	+	90 / 27.92	-	-	-	-	-	-	-	-
32	9,17-Octadecadienal	-	-	+	91 / 26.84	-	-	-	-	-	-
33	N-Hexadecanoic Acid	+	90 / 27.98	+	94 / 27.95	-	+	95 / 27.97	-	+	92 / 27.82
34	Ethyl 9-Hexadecenoate	+	91 / 26.40	-	-	-	-	-	-	-	-
35	9,12,15-Octadecatrienoic Acid, Methyl Ester	+	96 / 29.70	+	92 / 28.94	+	+	95 / 29.60	+	94 / 29.60	-
36	Phytol	+	95 / 29.78	+	96 / 29.79	+	+	95 / 29.79	+	96 / 29.78	96 / 29.77
37	Hexadecanoic Acid, Methyl Ester	+	95 / 28.20	+	96 / 27.25	-	-	-	+	96 / 27.25	-
38	Docosanoic Acid, Ethyl Ester	+	90 / 29.54	-	-	-	-	-	-	-	-
39	Tridecanoic Acid, 12-Methyl-, Methyl Ester	-	-	+	92 / 30.00	+	+	91 / 30.00	+	93 / 29.99	-
40	Ethanediamine,2,2-Oxybis[N,N-Dimethyl-]	-	-	-	-	-	+	91 / 31.98	+	91 / 31.98	92 / 31.98
41	Hexanedioic Acid, Bis(2-Ethylhexyl) Ester	+	93 / 33.25	-	-	-	-	-	-	-	-
42	9,12-Octadecadienoic Acid, Methyl Ester	+	93 / 29.60	+	95 / 29.61	-	-	-	+	94 / 29.60	95 / 29.60
43	Benzedrex	-	-	-	-	-	+	-	+	90 / 34.02	90 / 34.02
44	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	-	-	-	-	-	-	+	+	-	+

							90 / 34.83	90 / 37.05	91 / 34.84
45	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester	-	+	96 / 34.96	-	-	-	-	-
46	Octacosane	+	+	94 / 35.50	94 / 35.50	+	94 / 38.55	-	-
47	1-Eicosanol	-	+	91 / 36.67	-	-	-	-	+
48	Oxirane	-	-	-	-	-	-	-	93 / 28.13
49	Docosane	-	-	-	-	-	-	-	90 / 32.57
50	Trifluoroacetoxy hexadecane	-	-	-	-	-	-	-	90 / 36.54
51	Tetra triacontane	+	+	95 / 37.55	95 / 37.55	+	95 / 37.55	-	+
52	Squalene	+	+	96 / 37.76	96 / 37.76	+	97 / 37.76	97 / 37.76	92 / 36.65
53	1-Octacosanol	-	+	97 / 38.71	-	-	-	-	+
54	dl-tocopherol	-	-	-	-	-	-	-	95 / 41.01
55	Vitamin E	-	-	-	-	+	94 / 41.00	94 / 41.00	-
56	Campesterol	-	-	-	-	-	+	90 / 42.15	+
57	Stigmasterol	+	+	92 / 42.36	92 / 42.36	+	96 / 42.35	97 / 42.35	96 / 42.36
58	β-Sitosterol	+	+	93 / 42.92	93 / 42.92	+	93 / 42.91	92 / 42.92	+
59	Gamma-sitosterol	-	-	-	-	-	-	-	92 / 42.92

4.2 MTT assay results

Screening of potential anticancer agent is usually based on the evaluation of their level of potency and selectivity toward in vitro models. Potency is often expressed as GI₅₀ (concentration that inhibits cell growth by 50 %), TGI (concentration that totally inhibits cell growth) and IC₅₀ (lethal concentration resulting in 50 % of the cells) values (Boyd and Paull, 1995).

The cytotoxicity of the extracts on the colon 38 cells was determined by MTT assay. Figure 4.1 shows that the combination of three solvent extracts, Chloroform and the combination of chloroform with hexane extract, exhibits significant cytotoxic effects against colon cancer (colon 38) cells at concentrations of 6, 15 and 19 $\mu\text{g/mL}$ respectively ($P < 0.05$). However, all the tested extract except methanol and the mixture of methanol and chloroform, inhibited the growth of the tested cell line in a dose-dependent manner.

IC₅₀ is the concentration of extract that inhibits cell growth by 50 %. The IC₅₀ of the extracts calculated from the dose-response curves are shown in figure 4.2 The lower the bar, the better the activity, from the plot, the combination of three solvent extract showed a better activity (6 $\mu\text{g/mL}$) amongst all the tested extract followed by chloroform extract and then the combination of chloroform and hexane extract (15 and 19 $\mu\text{g/mL}$) respectively. No extract was able to inhibit the cancer cell as depicted by a no observed TGI value within the 100 $\mu\text{g/mL}$ concentration. Therefore, analysis is recommended using the combination of three solvent extract, chloroform extract and the combination of

chloroform and hexane. And also to examine the cytotoxicity effects of these extracts on normal cell line.

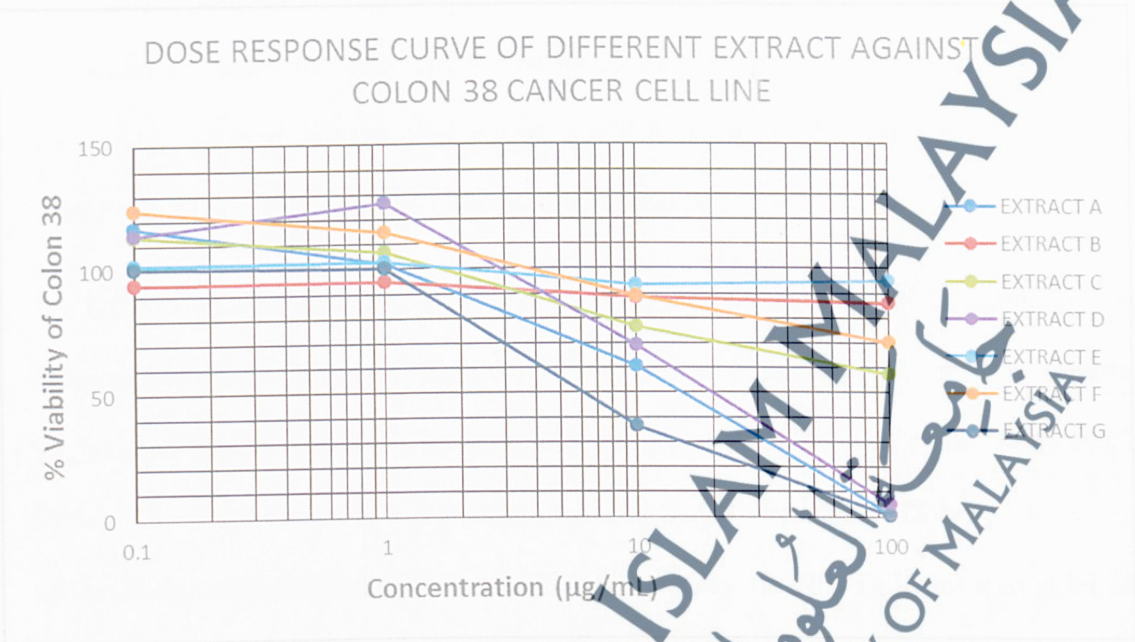


Figure 4.1: Effects of treatment with different extract on the viability of colon cancer cells evaluated through mitochondrial activity using MTT assay (A: Chloroform; B: Methanol; C: Hexane; D: Chloroform + hexane; E: Methanol + Chloroform; F: Methanol + hexane; G: 3 solvent).

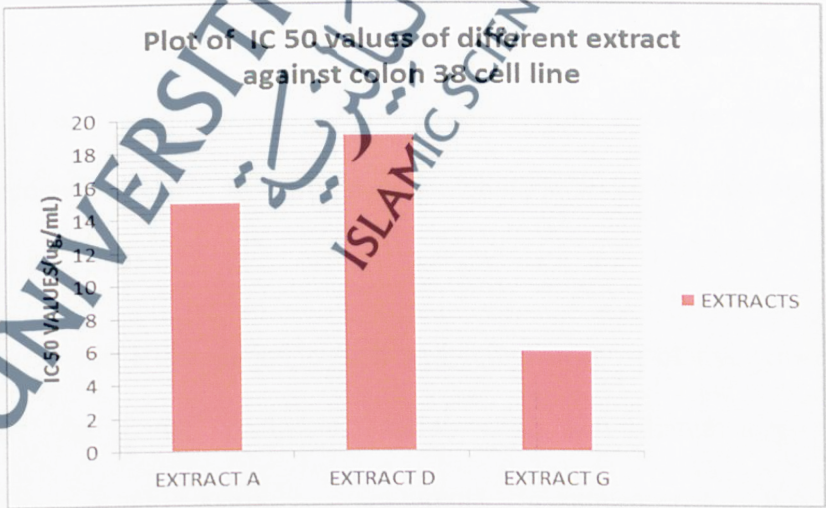


Figure 4.2: IC₅₀ of extracts A, D and G on colon 38 cell line after 72 hours.

4.3 Discussion

This study highlights the presence of many biochemical compounds in the aerial parts of *Clinacanthus nutans* that may affect pharmacological activities when used as medicine. This study indicated that the plant extract could be used to inhibit the growth of colon cancer cells. Thus, it is potential to be used as anticancer.

The GC-MS analysis of extracts showed the presence of various types of compounds in *Clinacanthus nutans* leaves. Certain compounds appear in specific solvent for example, Stigmasterol and β -Sitosterol were present in extraction using hexane; chloroform (50 %; 50 %). The Stigmasterol is a plant Sterol which has showed antimicrobial, anticancer, anti-arthritic, antiasthma diuretic and anti-inflammatory (Sudha, Chidambarampillai, & Mohan, 2013).

Hexadecanoic acid; ethyl ester is an ester of fatty acid which shows antioxidant activity, hypocholesterolemic, nematocide, hemolytic and 5-alpha reductase inhibitor (Cortés et al., 2014). The 9,12,15 Octadecatrienoic acid, ethyl ester is Linoleic acid ester, which showed many functions such as hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic 5-Alpha reductaseinhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne (Selvamangai & Bhaskar, 2012; Sudha et al., 2013).

Squalene is vitamin E compound, which was found in most of the extractions. It has reported as antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective,

hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic and anticoronary (Sudha et al., 2013).

Most of the compounds were identified in the absolute methanol fraction of *Clinacanthus nutans*, which are mainly amino, organic and phenolic acids, sugar and esters. These include Octadecanoic Acid; 2, 3-Dihydroxypropyl Ester; Benzedrex; β -D-Glucopyranoside; Methyl, β -D-Glucopyranose; 1,6-Anhydro, 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl; and Undecane. These major compounds found in *C. nutans* not only are lipophilic but also exhibited antimicrobial activity (Yong et al., 2013a).

Other compound which was present in all extracts was phytol (acyclic diterpene alcohol) and its derivative 3,7,11,15-Tetramethyl-2-Hexadecan-1-OL. Phytol is used as antimicrobial and anti-inflammatory, is effective in different stages of arthritis. It also acts as anticancer and diuretic (Sudha et al., 2013).

The mixture of the three solvents exhibited the potential anticancer activity compared to the other extracts. The possible explanation of this is that most of these compounds are semi polar by mixing the polar and non-polar solvents (methanol, hexane and chloroform). This mixture of the three solvents was able to dissolve these compounds and capable to extract them, contributing to the anticancer activity. The entire compounds extracted by solvent containing chloroform showed significant anticancer activity. This is in agreement with the study on *C. nutans* leaves extracted by chloroform, which showed the most potent phytochemical constituents and the highest inhibition activity on cancer cell lines compared to water and methanol extracts (Yong et al., 2013a). However, there

is no study yet examined the effect of solvent mixture on the phytochemical constituents by GC-MS and anticancer activity as it has been done in this research.

