CHAPTER 4

RESULTS

4.1 Yield of Crude Extracts

Forty species of vegetables were extracted using two solvents systems namely, methanol and water. The yield of extracts obtained from methanol and water extraction are shown in Table 4.1.

The total yield percentages range from 5.72% to 48.78%. The *Lycopersicum esculentum* showed the highest total yield percentage while *Centella asiatica* showed the lowest. Generally, the yield percentages of the crude methanol extracts (ranged 1.74% to 37.44%) were higher than that of the water extracts (ranged 2.01% to 27.90%).

Table 4.1 Yield of crude extracts obtained from selected vegetable species

No	Plant	Extraction Solvent	Weight (g)	Yield (%)	Total yield (%)
1	*Asparagus officinalis*	Methanol Water	3.23 3.82	6.16 9.10	15.26
2	*Allium fistulosum*	Methanol Water	2.71 3.01	13.58 15.08	28.66
3	*Allium tuberosum*	Methanol Water	0.96 1.09	4.83 5.48	10.32
4	*Capsicum annum*	Methanol Water	4.92 3.16	24.63 15.81	40.44
5	*Capsicum frutescens*	Methanol Water	0.50 0.95	2.50 4.79	7.30
6	*Lycopersicum esculentum*	Methanol Water	7.48 2.26	37.44 11.34	48.78
7	*Solanum melongena*	Methanol Water	4.15 3.01	20.79 15.07	35.87
8	*Solanum tuberosum*	Methanol Water	2.61 1.09	13.08 5.46	18.55
9	*Cucumis sativus*	Methanol Water	3.16 2.78	15.83 13.93	29.76

Table 4.1 Continued

10	*Cucurbita moschata*	Methanol Water	4.57 4.94	22.89 24.73	47.63
11	*Lagenaria sceraria*	Methanol Water	2.36 1.05	11.84 5.25	17.10
12	*Momordica charantia*	Methanol Water	3.37 3.10	16.89 15.50	32.40
13	*Luffa acutangula*	Methanol Water	1.31 2.11	6.56 10.59	17.15
14	*Cosmos caudatus*	Methanol Water	1.39 1.34	1.39 1.34	13.70
15	*Lactuca sativa*	Methanol Water	2.31 1.94	2.31 1.94	21.32
16	*Archidendron jiringga*	Methanol Water	1.66 1.51	1.66 1.51	15.89
17	*Neptunia prostrata*	Methanol Water	1.61 1.51	1.61 1.51	15.66
18	*Pachyrrhizus erosus*	Methanol Water	5.61 5.58	5.61 5.58	55.98
19	*Psophocarpus tetragonolobus*	Methanol Water	0.60 1.00	0.60 1.00	8.03
20	*Parkia speciosa*	Methanol Water	3.82 2.20	3.82 2.20	30.14
21	*Sesbania grandiflora*	Methanol Water	2.98 2.35	2.98 2.35	26.71
22	*Vigna sinensis*	Methanol Water	1.05 2.09	1.05 2.09	15.76
23	*Pisum sativum*	Methanol Water	1.68 2.76	8.40 13.82	22.22
24	*Phaseolus vulgaris*	Methanol Water	0.98 1.67	4.90 8.39	13.30
25	*Amaranthus gangeticus*	Methanol Water	1.65 2.77	8.25 13.86	22.11
26	*Amaranthus viridis*	Methanol Water	0.87 3.77	4.36 18.87	23.23
27	*Hibiscus esculentus*	Methanol Water	3.95 0.87	19.77 4.35	24.12
28	*Averrhoa carambola*	Methanol Water	1.67 0.91	8.37 4.56	12.93
29	*Manihot esculenta*	Methanol Water	0.38 2.79	1.94 13.95	15.90
30	*Portulaca oleracea*	Methanol Water	2.37 1.44	11.86 7.21	19.07
31	*Ipomoea batatas*	Methanol Water	2.91 2.77	14.58 13.89	28.47

Table 4.1 Continued

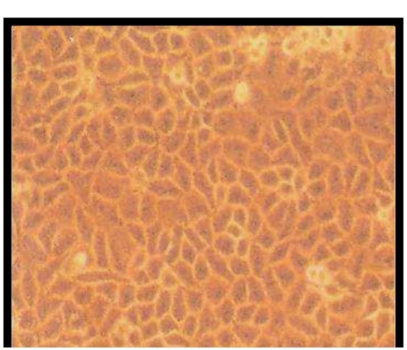
32	*Ipomoea reptans*	Methanol Water	2.77 2.61	13.89 13.08	26.97

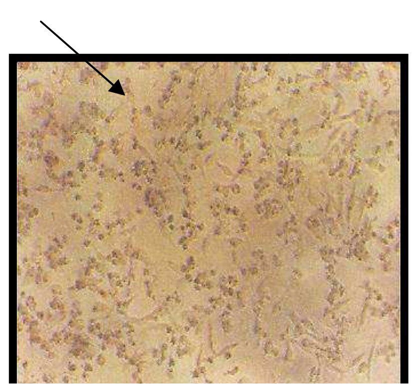
33	*Apium graveolens*	Methanol Water	1.05 1.07	5.29 5.39	10.68
34	*Centella asiatica*	Methanol Water	0.70 0.40	3.50 2.011	5.72
35	*Daucus carota*	Methanol Water	4.76 1.00	23.82 5.01	28.84
36	*Oenanthe javanica*	Methanol Water	3.16 2.62	15.80 13.10	28.91
37	*Beta vulgaris*	Methanol Water	2.61 1.95	13.07 9.76	22.83
38	*Citrus aurantifolia*	Methanol Water	1.98 1.39	9.93 6.97	16.91
39	*Brassica oleracea* var. *alboglabra*	Methanol Water	1.98 2.49	9.93 12.48	22.41
40	*Petroselium crispum*	Methanol Water	0.34 3.38	1.74 16.92	18.67

4.2 Screening For The *In vitro* Cytotoxic Activity of Crude Methanol and Water Extracts of Selected Vegetables

In the present study, 80 crude methanol and water vegetable extracts were evaluated for their *in vitro* cytotoxic activity against two cervical cancer-derived cell lines (HeLa and CaSki) and human fibroblast cell line (MRC5) using the neutral red cytotoxic assay. HeLa and CaSki cells were grown in 10% supplemented RPMI 1640 medium and MRC5 cells grown in 10% supplemented EMEM medium were incubated with methanol and water extracts of selected vegetables at varying concentrations (1, 10, 25, 50, 100 μg/ml) for 72 hours in a 5% CO_2 incubator at 37ºC. The negative controls consisted of cells not treated with vegetable extracts. Figure 4.1 shows human cancer cell lines, HeLa, CaSki and MRC5 cells treated with 100 μg/ml of vegetables crude extracts. The negative controls exhibited normal proliferation rate and showed no sign of death after the incubation time of 72 hours, while HeLa, CaSki and MRC5 cells treated with 100 μg/ml of vegetables crude extracts exhibited decrease in proliferation rate and showed signs of death after the incubation period of 72 hours.

The cytotoxicity results obtained were presented as inhibition percentages relative to the negative control and the IC_{50} values were extrapolated from the dose response curves plotted from the percentage of inhibition values. The IC_{50} value is the concentration of extract that inhibits the growth 50% of cells. An extract which gives IC_{50} value of 20 μg/ml and below is considered cytotoxically active (Geran *et al*., 1972; Chiang *et al*., 2003).





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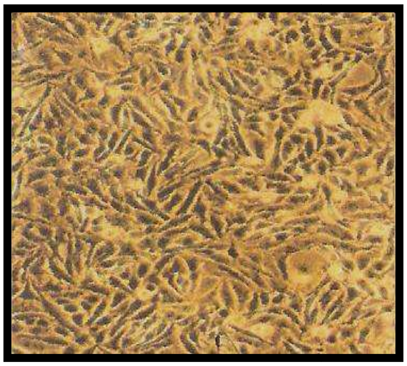
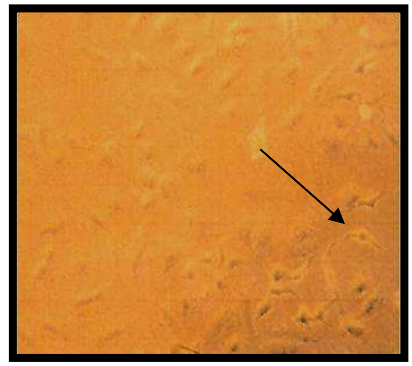
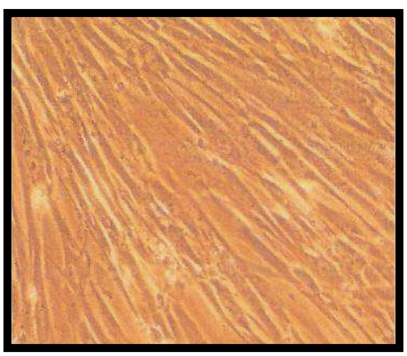
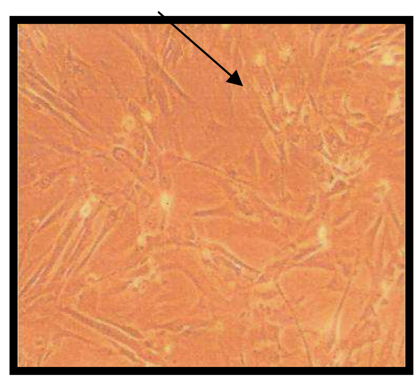




(c) (d)





(e) (f)

Figure 4.1: Photomicrograph (100X) of: (a): Untreated CaSki cells incubated (negative contol) (b): CaSki cell treated with 100 µg/ml of crude extract of *Beta vulgaris* (c): untreated HeLa cells incubated (negative control) (d) CaSki cell treated with 100 µg/ml of crude extract of *Portulaca oleracea* (e): untreated MRC5 cells incubated in EMEM (Negative control) (f): MRC5 cells treated with 100 µg/ml of crude MeoH extract of *Capsicum annum*

4.2.1 Cytotoxic Activity of Doxorubicin Against HeLa and CaSki Cells and MRC5 Cell Lines

The human cervical cancer-derived cell lines, (HeLa and CaSki) and human fibroblast cell line (MRC5), were treated with doxorubicin, a positive control for the neutral red cytotoxic assay.

Cells treated with doxorubicin exhibited decreased proliferation rate and showed signs of death after the incubation of 72 hours. Results showed that the doxorubicin shown significant cytotoxic effects against HeLa, CaSki and MRC5 cells (Table 4.2, Table 4.3, Figure 4.2). Doxorubicin to be cytotoxic of all cell lines tested in a dose-dependent manner with inhibition percentages of 5.89-44.99% at $1x10^{-5}$ µg/ml, 13.08-45.46% at $1x10^{-4}$ µg/ml, 23.86-47.68% at $1x10^{-3}$ µg/ml, 46.81-69.91% at $1x10^{-2}$ µg/ml, 46.95-76.36% at $1x10^{-1}$ µg/ml, 50.91-84.54% at 1x10 µg/ml, 53.10-87.82% at $1x10^{1}$ µg/ml and 63.39-89.48% at $1x10^{2}$ µg/ml.

The inhibition percentages obtained were presented as dose-respone curves. The *in vitro* growth inhibition of doxorubicin against HeLa, CaSki and MRC5 cell lines are shown in Figure 4.2. The IC_{50} values were estimated from the graph extrapolation and summarized in Table 4.3. The IC_{50} value refers to the effective dose (concentration of extracts in µg/ml) that inhibits 50% of cells growth. Extracts having an IC_{50} value equal to or less than 20 are considered active for cytotoxic assay against cells (Geran *et al*., 1972).

Table 4.2: Inhibition of HeLa, CaSki and MRC5 cells treated with doxorubicin

Cell line	Percentage of inhibition ± standard deviation (%) at different concentration (μg/ml)							
	$1x10^{-5}$	**$1x10^{-4}$**	**$1x10^{-3}$**	**$1x10^{-2}$**	**$1x10^{-1}$**	**1**	**10**	**100**
HeLa	20.1±5.5	21.6±9.4	26.5±1.7	46.8±2.8	47.0±0.9	53.6±0.5	55.3±0.7	63.3±1.3
CaSki	5.9±3.7	13.1±1.3	23.9±0.5	69.9±1.5	76.4±0.5	84.5±0.2	87.8±0.4	89.5±0.4
MRC5	45.0±0.12	45.5±0.6	47.7±0.4	48.3±0.3	49.0±0.5	50.9±0.5	53.1±0.4	68.4±1.4

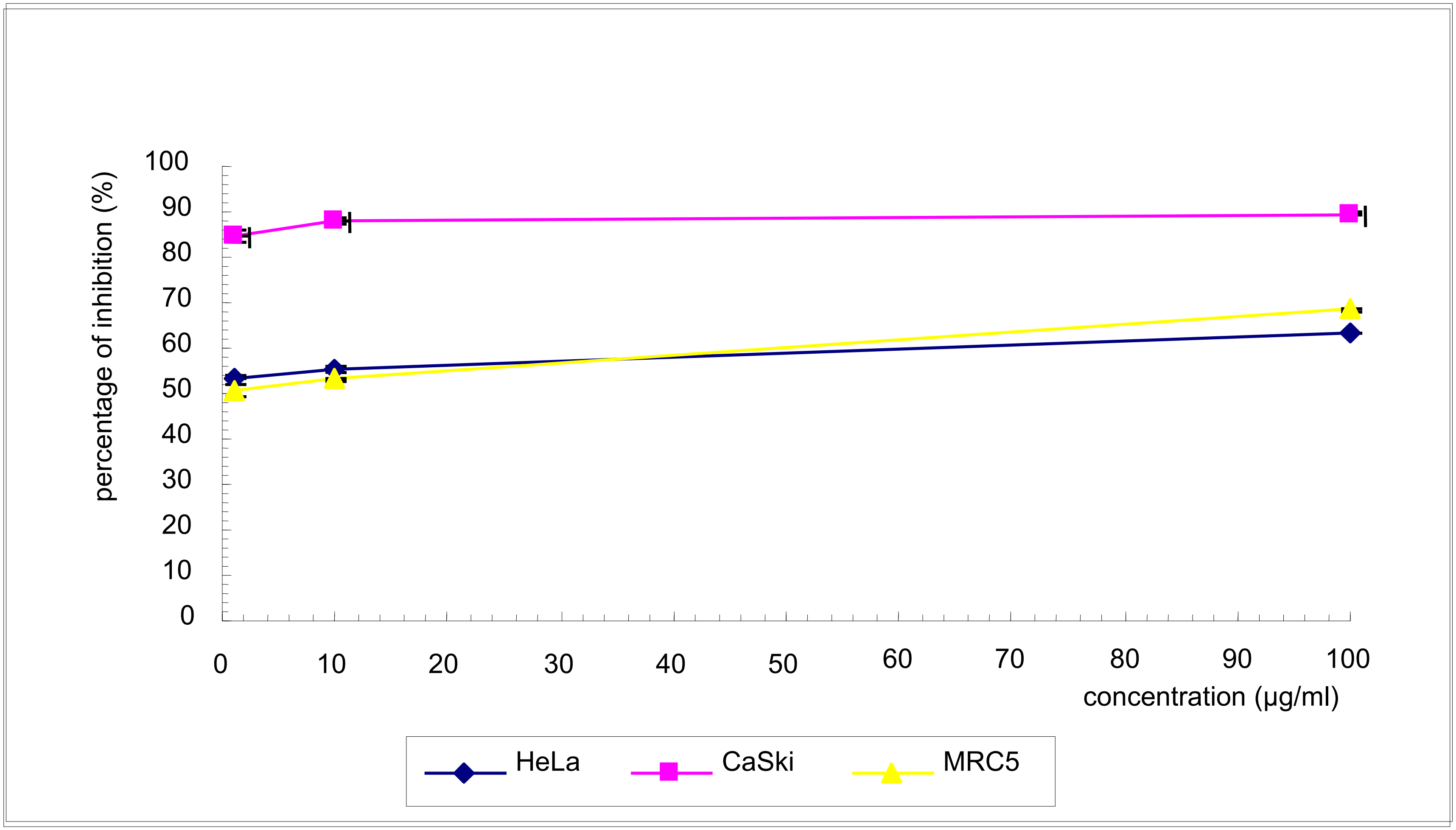


Figure 4.2: Dose-response curve showing *in vitro* growth inhibition of HeLa, CaSki and MRC5 cells by doxorubicin (positive control)

Table 4.3: The IC_{50} values of doxorubicin against various cell lines

Cell lines	IC_{50} value (µg/ml)
HeLa	3.00
CaSki	0.05
MRC5	4.00

4.2.2 *In vitro* Cytotoxic activity of Selected Vegetable Extracts Against HeLa Cells

The *in vitro* growth inhibition of HeLa cells by crude methanol extracts of the 40 vegetables are shown as dose-response curves in Figures 4.3(a) to 4.3(h). The IC_{50} values were extrapolated from the dose-response curves and summarized in Table 4.4. The inhibition activities ranged from 4.57-41.36% at 1µg/ml, 9.7-65.66% at 10 µg/ml, 14.95-81.93% at 25 µg/ml, 23.65-84.92 at 50 µg/ml and 28.17-87.78 at 100 µg/ml. Out of 40 vegetables evaluated only 7 vegetables (17.5%) methanol extracts, namely *Capsicum annum (lada merah), Capsicum frutescent (cili padi)s, Lycopersicum esculentum (tomato), Cosmos caudatus (ualm rajah), Vigna sinensis (kacang panjang), Amaranthus viridis (bayam merah)* and *Ipomoea reptans* (*kangkung*) were actively cytotoxic against HeLa cell with IC_{50} values less than 20 µg/ml. The IC_{50} values are 13 µg/ml, 17.5 µg/ml, 17.4 µg/ml, 18.02 µg/ml, 15.9 µg/ml, 15 µg/ml and 5.9 µg/ml, respectively.

Crude methanol extracts of *Asparagus officinalis (asparagus), Solanum tuberosum (ubi kentang), Cucumis sativus (timun), Cucurbita moschata (labu merah), Lagenaria sceraria (labu ayer), Momordica charantia (peria), Luffa acutangula (petola segi),* *Lactuca sativa (salada), Neptunia prostrate (tangki), Pachyrrizus erosus (sengkuwang), Parkia speciosa (petai), Amaranthus gangeticus (bayam pasir), Apium graveolens (selderi), Centella Asiatica (Pegaga), Daucus carota (lobak), Beta vulgaris (bit), Citrus aurantifolia (limau), Pisum sativum (kacang putih), Brassica oleracea* var *alboglabra (kalian), Phaseolus vulgaris kacang buncis),* and *Petroselum crispum (parsley)* possessed inhibited HeLa cells with percentage inhibition less than 50% at all concentrations tested in this study. Therefore, IC_{50} values could not be determined from the existing dose-response curve

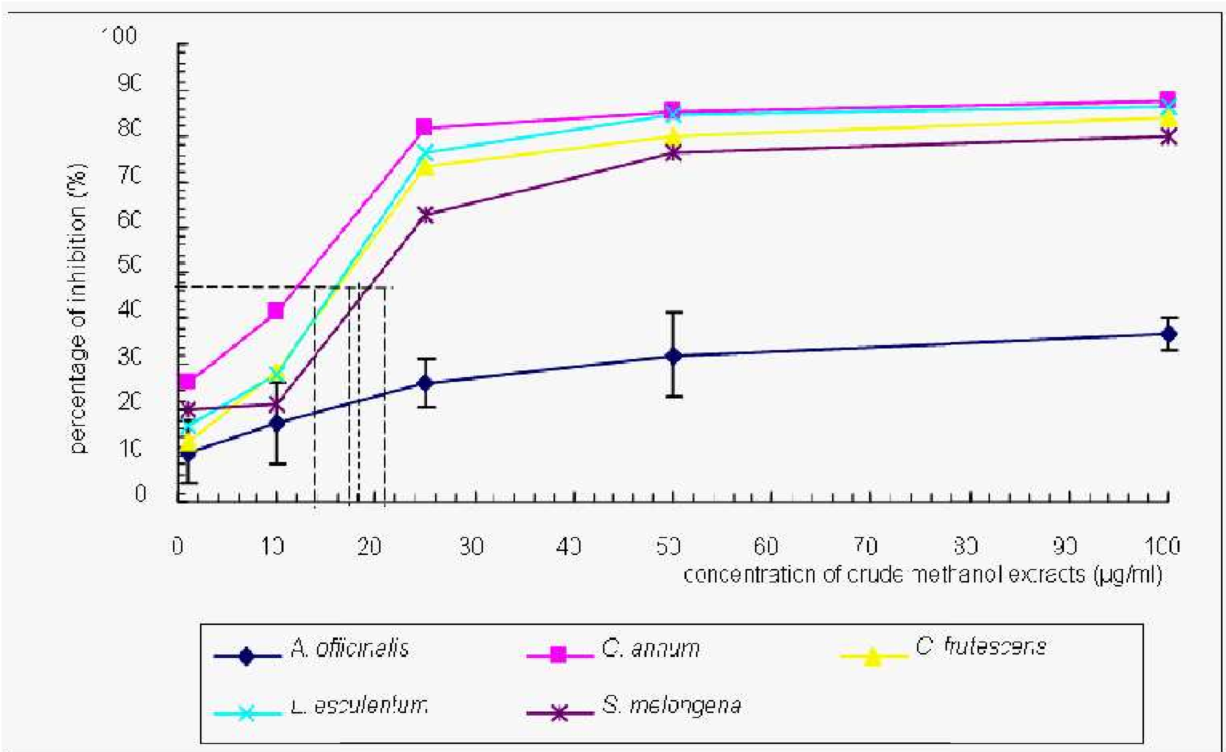


Figure 4.3(a): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against HeLa cells.

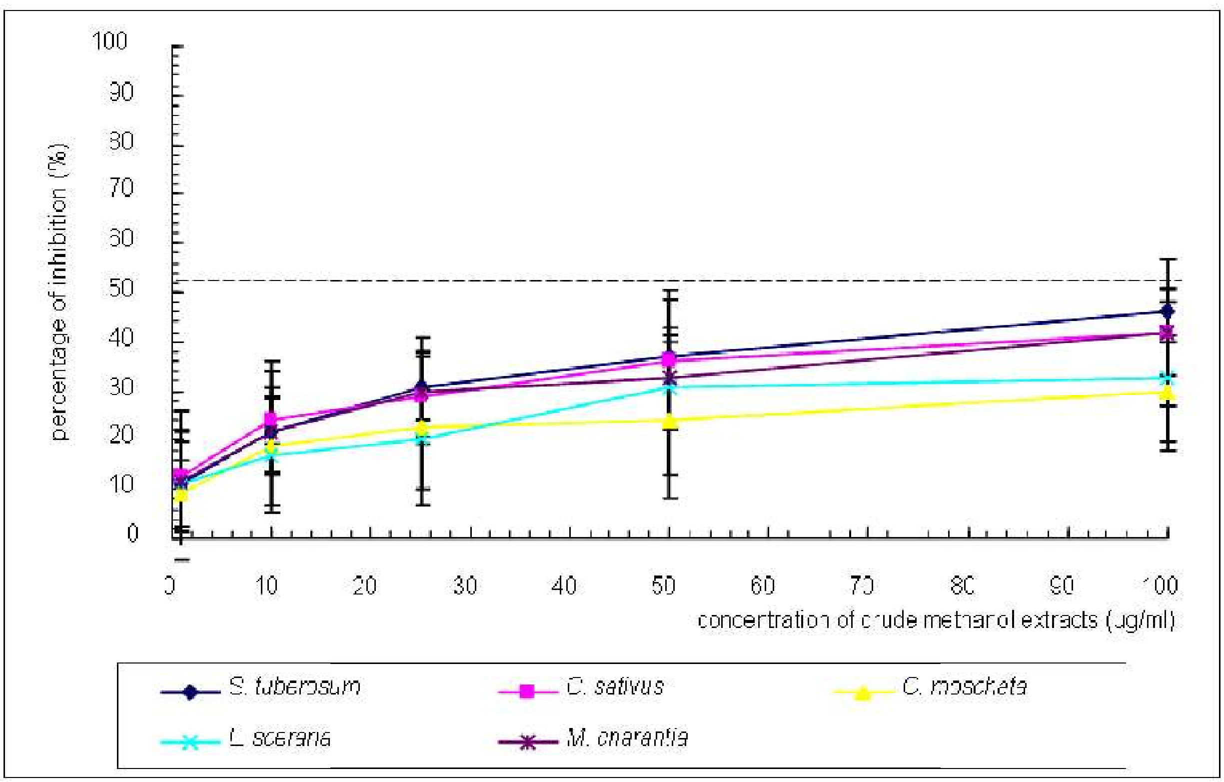


Figure 4.3 (b): Dose-response curves showing cytotoxic activity of crude methanol extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against HeLa cells.

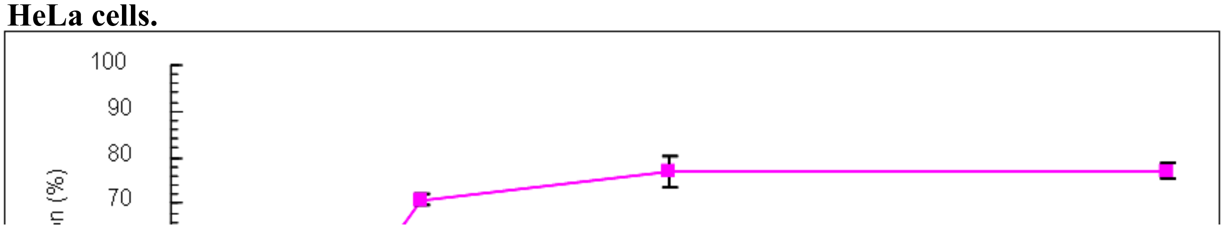


Figure 4.3 (c): Dose-response curves showing cytotoxic activity of crude methanol extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrate* against HeLa cells.

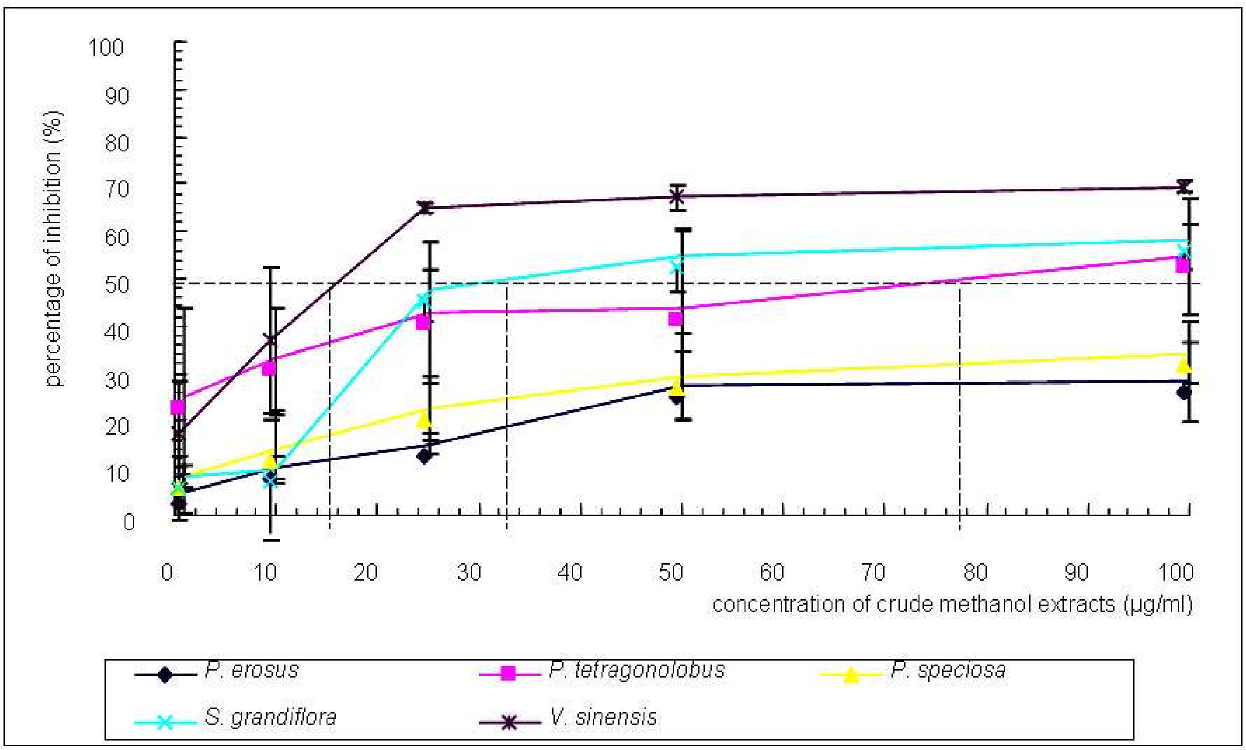


Figure 4.3 (d): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against HeLa cells.

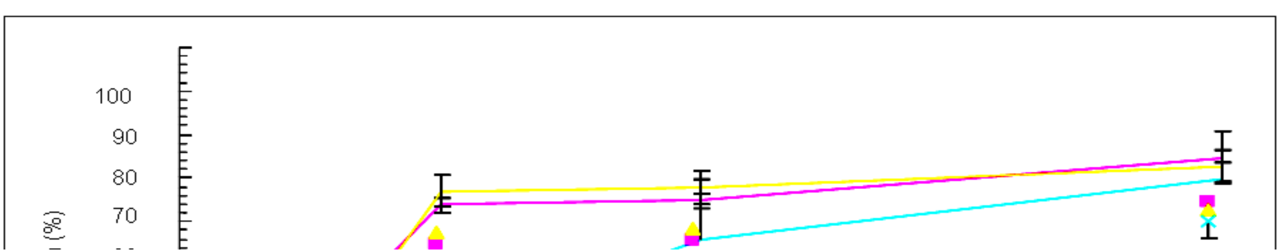


Figure 4.3 (e): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. gangeticus, A. viridis, H. esculenthus, A. carambola* and M. esculenta against HeLa cells.

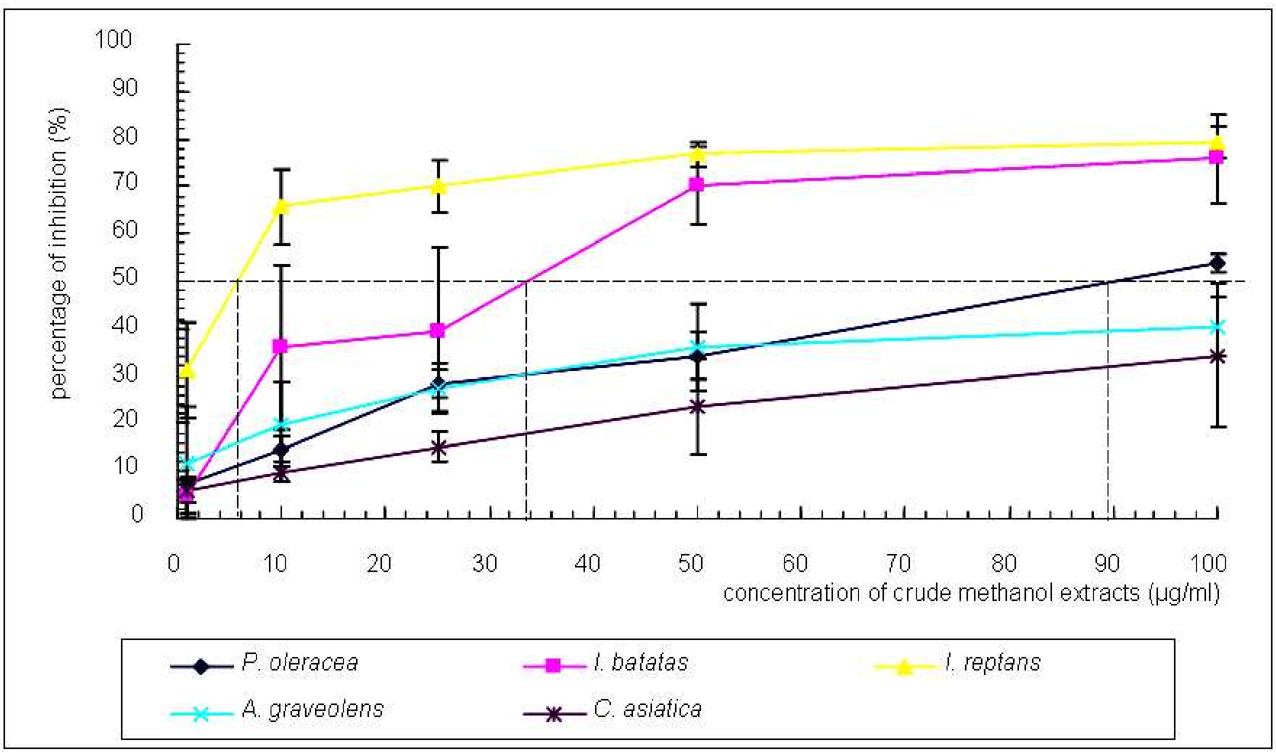


Figure 4.3 (f): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against HeLa cells.

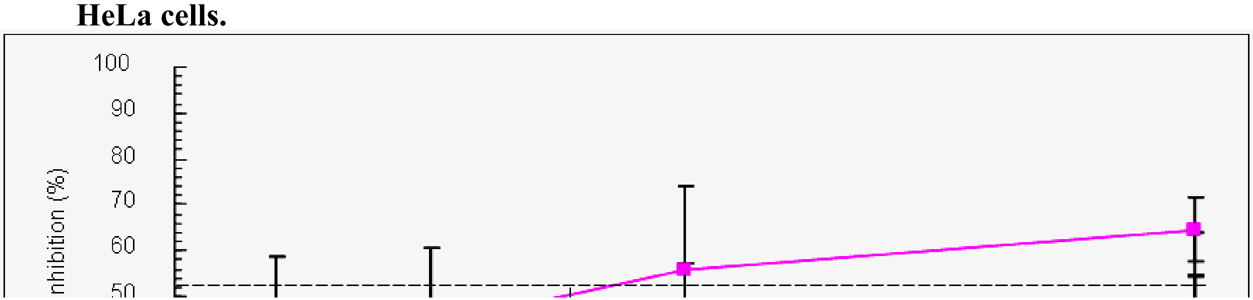


Figure 4.3 (g): Dose-response curves showing cytotoxic activity of crude methanol extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against HeLa cells.

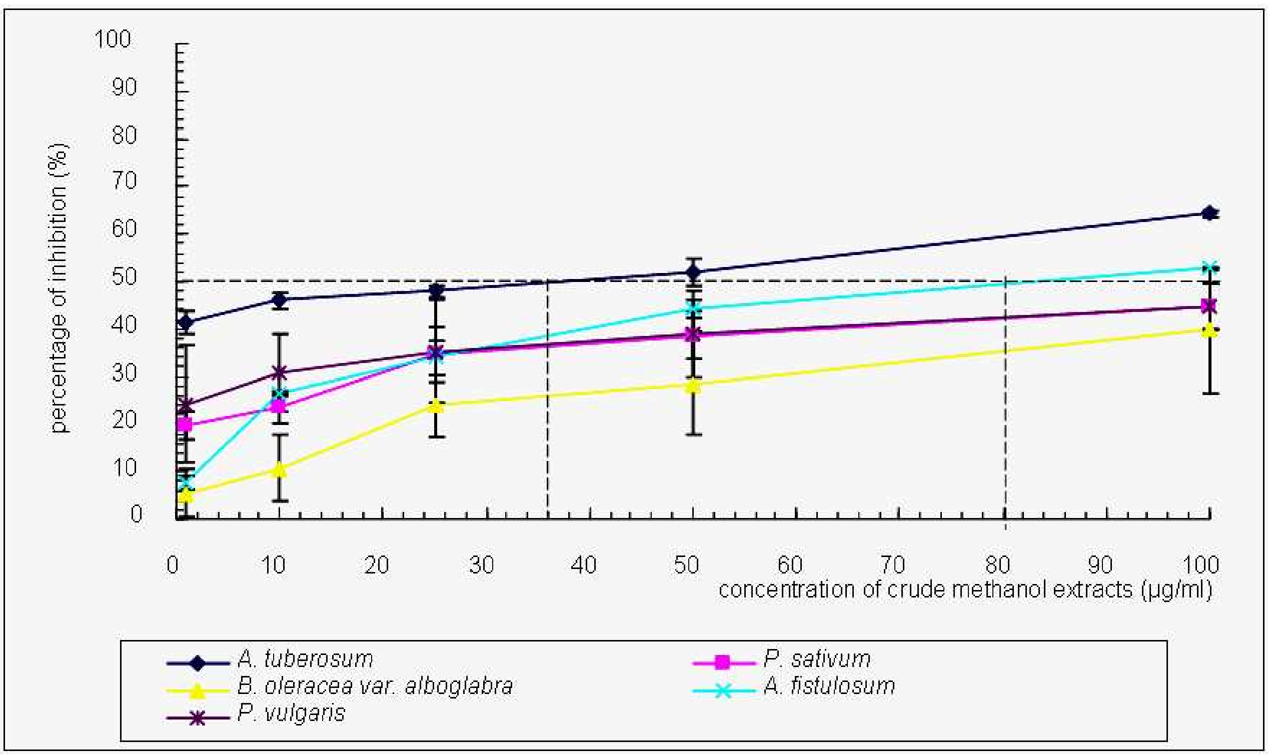


Figure 4.3 (h): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. tuberosum, P. sativum, B. oleracea var. alboglabra* and *P. vulgaris* against HeLa cells

Table 4.4: IC_{50} values of crude methanol extracts of selected vegetables against HeLa cells

No	Plant	Local name	IC_{50} (µg/ml)
1	*Ipomoea reptans*	kangkung	**5.90**
2	*Capsicum annum*	lada merah,	**13.00**
3	*Amaranthus viridis*	bayam pasir	**15.00**
4	*Vigna sinensis*	kacang panjang	**15.90**
5	*Hibiscus esculentus*	bendi	**17.00**
6	*Lycopersicum esculentum*	tomato	**17.40**
7	*Capsicum frutescens*	chabai, lada api, lada kerawit	**17.50**
8	*Cosmos caudatus*	ulam rajah	**18.02**
9	*Solanum melongena*	terong	20.05
10	*Averrhoa carambola*	belimbing besi	32.00
11	*Sesbania grandiflora*	turi	33.50
12	*Ipomoea batatas*	keledek	33.80
13	*Allium tuberosum*	Ku chai	36.00
14	*Oenanthe javanica*	shelum	38.50
15	*Manihot esculenta*	ubi kayu	39.00
16	*Archidendron jiringga*	jering	64.00
17	*Psophocarpus tetragonolobus*	kacang botor,	78.00
18	*Allium fistulosum*	Daun bawang	80.00
19	*Portulaca oleracea*	beremi	89.50
20	*Asparagus officinalis*	Asparagus, saparu keras	>100
21	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	>100

Table 4.4 continued

22	*Cucumis sativus*	timun, timun China, mentimun	>100
23	*Cucurbita moschata*	labu merah, labu perang,	>100
24	*Lagenaria sceraria*	labu ayer putih, labu botol	>100
25	*Momordica charantia*	peria	>100
26	*Luffa acutangula*	petola segi, ketola segi	>100
27	*Lactuca sativa*	salad, selada	>100
28	*Neptunia prostrata*	tangki,	>100
29	*Pachyrrhizus erosus*	sengkuwang,	>100
30	*Parkia speciosa*	petai	>100
31	*Pisum sativum*	kacang puteh	>100
32	*Phaseolus vulgaris*	kacang buncis	>100
33	*Amaranthus gangeticus*	bayam merah	>100
34	*Apium graveolens*	selderi	>100
35	*Centella asiatica*	pegaga	>100
36	*Daucus carota*	lobak merah	>100
37	*Beta vulgaris*	bit	>100
38	*Citrus aurantifolia*	limau	>100
39	*Brassica oleracea* var. *alboglabra*	Kai lan	>100
40	*Petroselium crispum*	parsley	>100

The *in vitro* growth inhibition of HeLa cells by crude water extracts of the 40 selected vegetables are shown as dose-response curves in Figures 4.4(a) to 4.4(h). The inhibition rates of HeLa cells were 2.00-48.47% at 1 µg/ml, 7.57-51.47 at 10 µg/ml, 11.33-64.44% at 25 µg/ml, 11.63-67.63% at 50 µg/ml and 12.57-72.15% at 100 µg/ml. Only 2 out of 40 crude water extracts of the selected vegetables studied namely, *C. annum* and *L. sceraria* exhibited IC_{50} values less than 20 µg/ml and therefore considered actively cytotoxic against HeLa cells (Table 4.5). When compared *L. sceraria* were the more active. The IC_{50} value for crude water extracts of *L. sceraria* was 6 µg/ml while the IC_{50} value for crude water extracts of *C. annum* was 17 µg/ml. On the other hand, *C. frutescens*, *L. esculentum, C. moschata, C. caudatus* and *V. sinensis, A . fistulosum, S. grandiflora, A. graveolens* and *C. aurantifolia* exhibited no cytotoxic rates against HeLa cells. Their IC_{50} values ranged from 39.5 µg/ml to 90 µg/ml. The remainder of water extracts exhibited IC_{50} values greater than 100 µg/ml against HeLa cells.

Generally, from 80 crude methanol and water extracts of selected vegetables studied, only 9 of them exerted active cytotoxic effect against HeLa cells. Both crude methanol and water extracts of *C. annum* exhibited the active cytotoxic effects against HeLa cells with IC_{50} values of 13 µg/ml and 17 µg/ml, respectively. The crude methanol extracts of *I. reptans* and the crude water extracts of *L. sceraria* appeared to be more potent as compared to the others due to their abilities to retard more than 50% of cell growth at concentrations lower than as 10wer than 10 µg/ml.

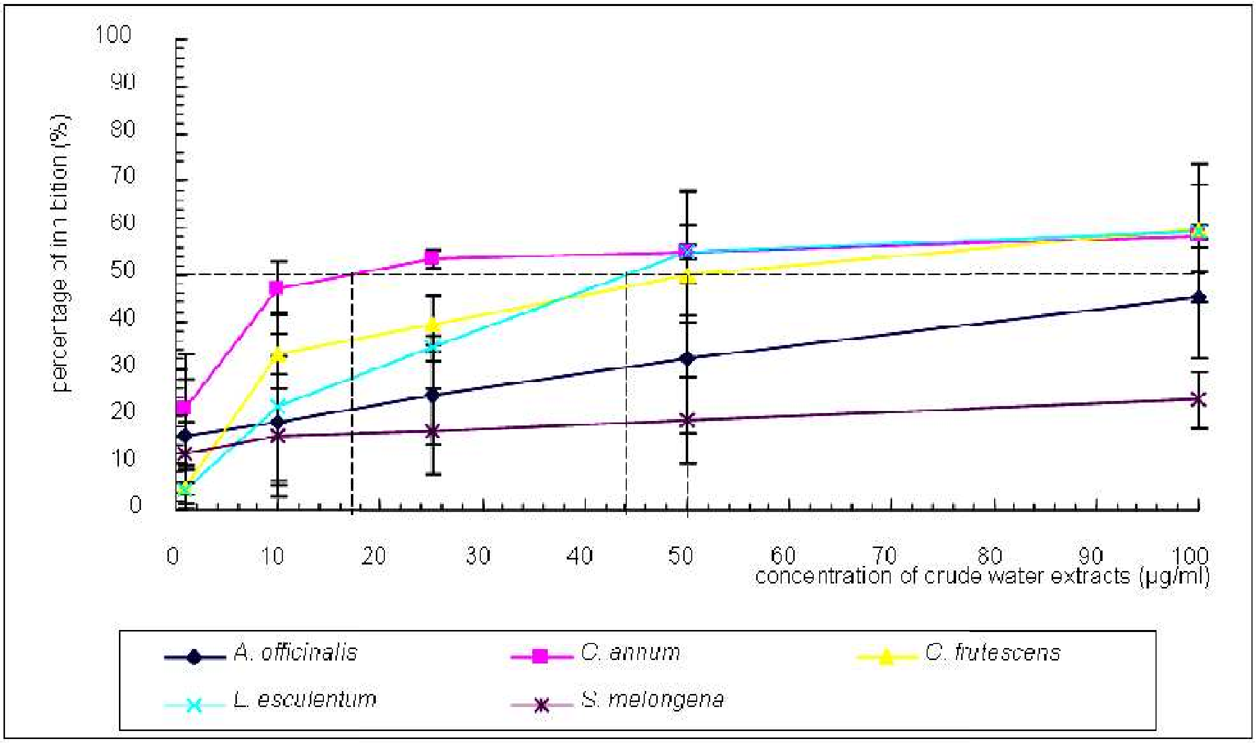


Figure 4.4(a): Dose-response curves showing cytotoxic activity of crude water extracts of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against HeLa cells.

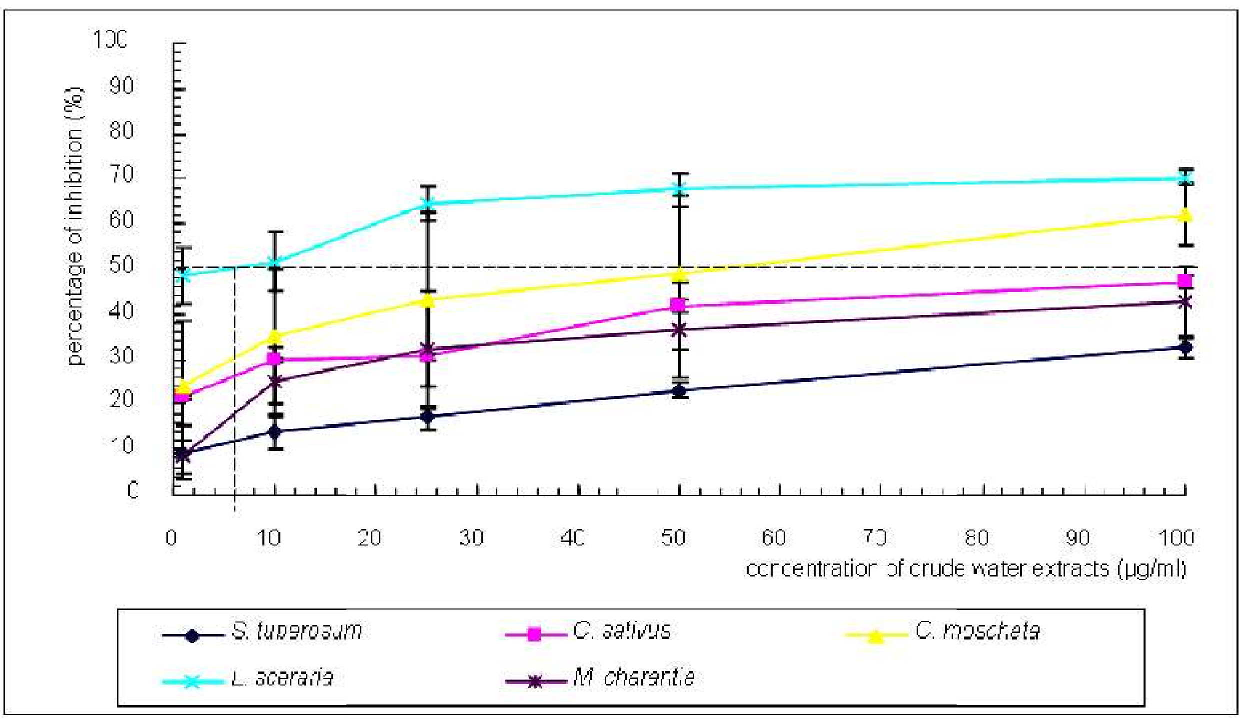


Figure 4.4 (b): Dose-response curves showing cytotoxic activity of crud water extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against HeLa cells.

Figure 4.4 (c): Dose-response curves showing cytotoxic activity of crude water extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrate* against HeLa cells.

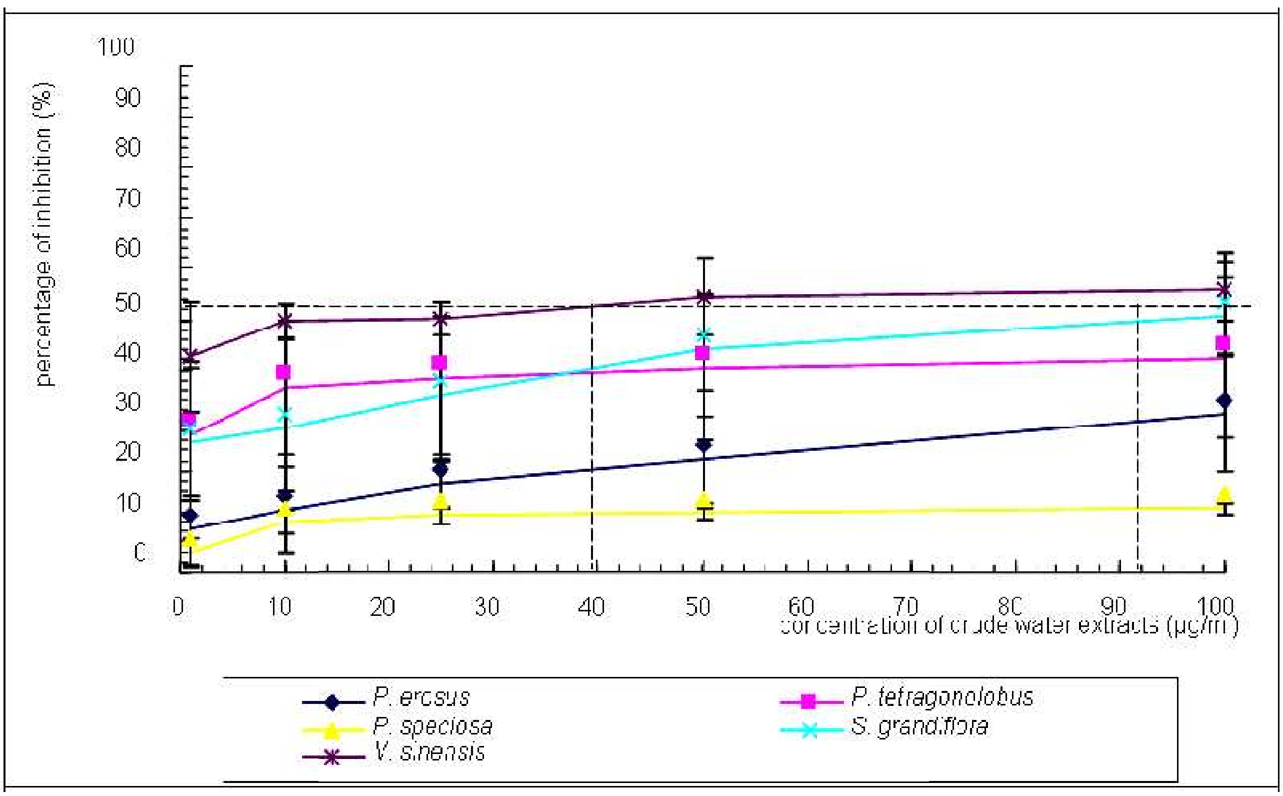


Figure 4.4 (d): Dose-response curves showing cytotoxic activity of crude water extracts of *P. erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against HeLa cells.

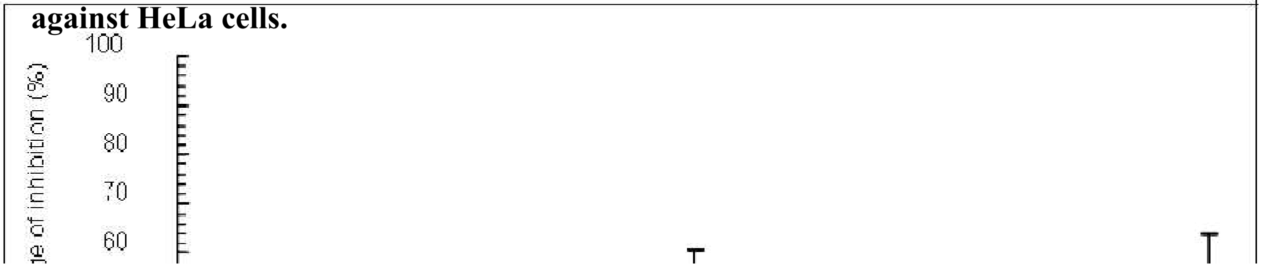


Figure 4.4 (e): Dose-response curves showing cytotoxic activity of crude water extracts of *A. gangeticus, A. viridis, H. esculenthus, A. carambola* and M. esculenta against HeLa cells.

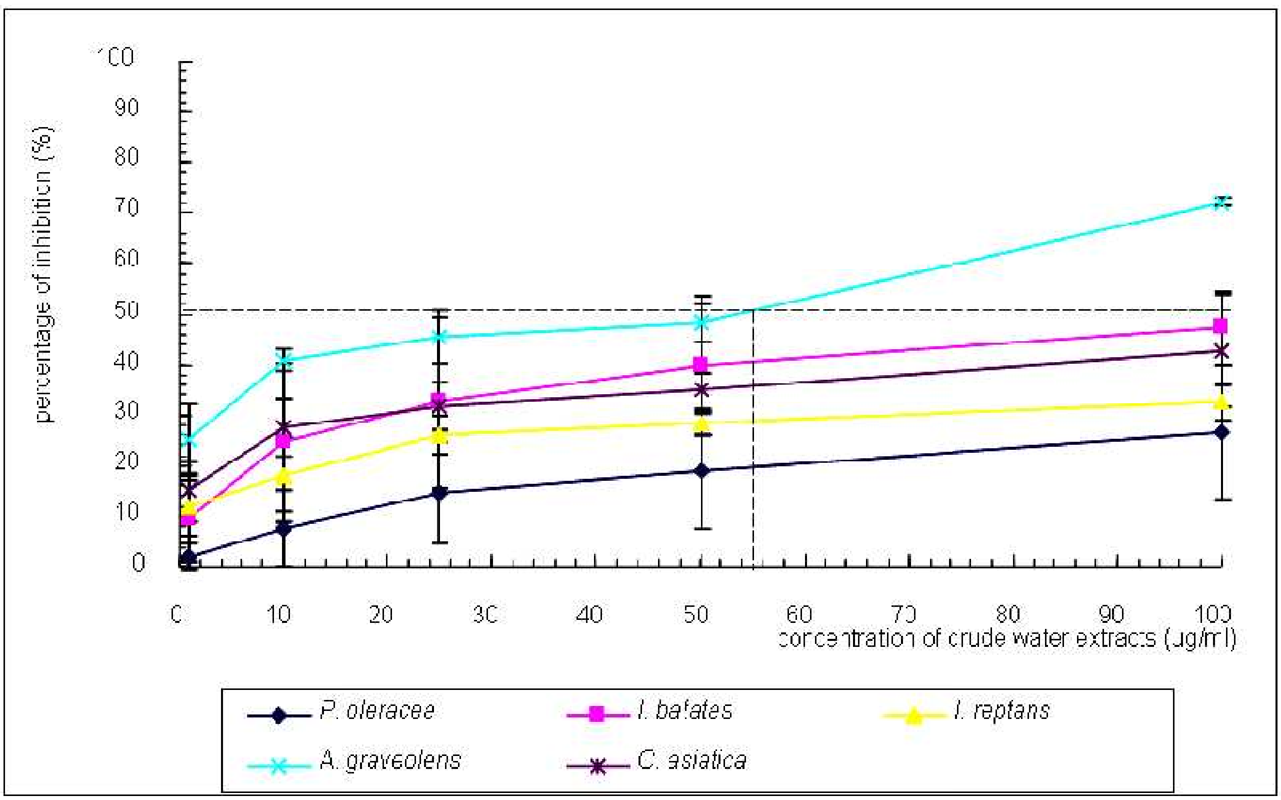


Figure 4.4 (f): Dose-response curves showing cytotoxic activity of crude water extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against HeLa cells.

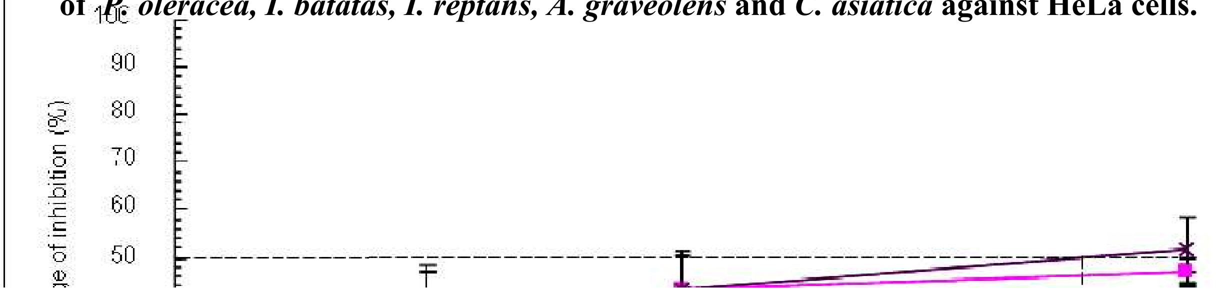


Figure 4.4 (g): Dose-response curves showing cytotoxic activity of crude water extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against HeLa cells.

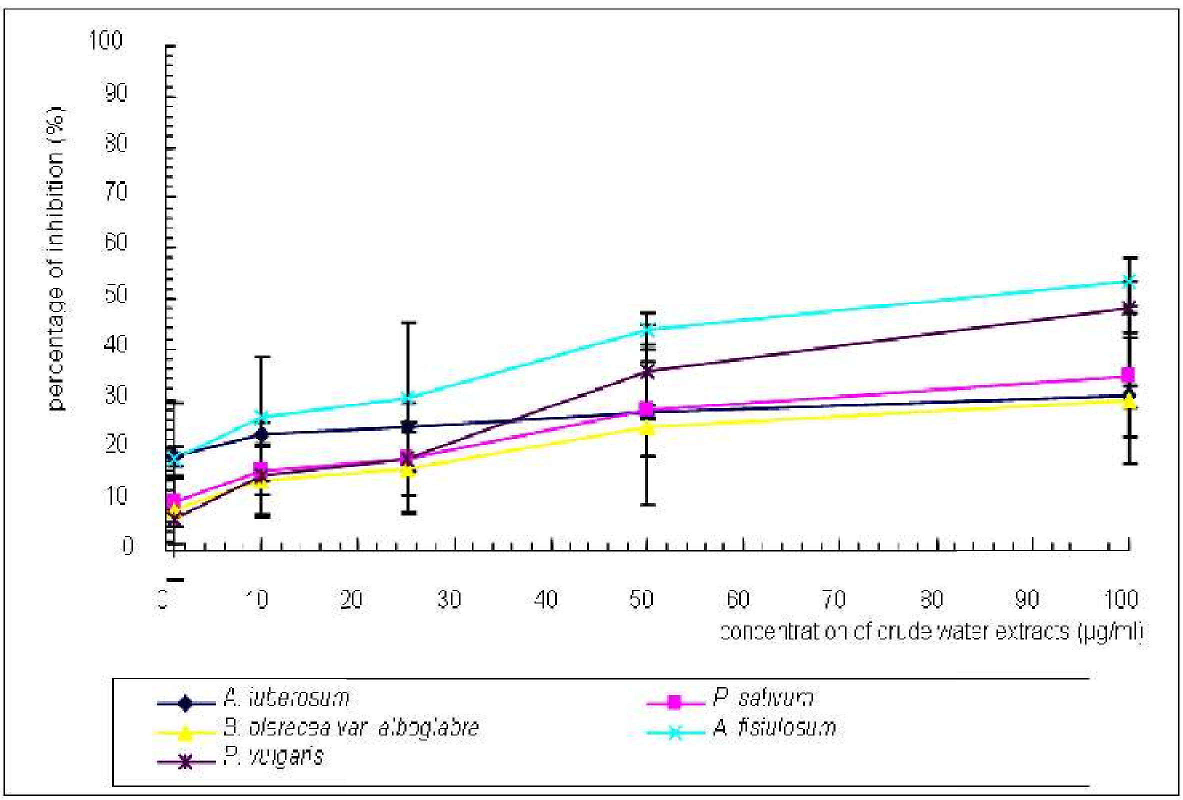


Figure 4.4 (h): Dose-response curves showing cytotoxic activity of crude water extracts of *A. tuberosum, P. sativum, B. oleracea var. alboglabra* and *P. vulgaris* against HeL

Table 4.5: IC_{50} values of crude water extracts of selected vegetables against HeLa cells

No	Plant	Local name	IC_{50} (µg/ml)
1	*Lagenaria sceraria*	labu ayer putih, labu botol	**6.00**
2	*Capsicum annum*	lada merah,	**17.00**
3	*Vigna sinensis*	kacang panjang	39.50
4	*Lycopersicum esculentum*	tomato	44.00
5	*Capsicum frutescens*	chabai, lada api, lada kerawit	50.00
6	*Cosmos caudatus*	ulam rajah	50.00
7	*Cucurbita moschata*	labu merah, labu perang,	54.50
8	*Apium graveolens*	selderi	55.00
9	*Allium fistulosum*	Daun bawang	80.00
10	*Sesbania grandiflora*	turi	90.00
11	*Citrus aurantifolia*	limau	90.00
12	*Asparagus officinalis*	Asparagus, saparu keras	>100
13	*Allium tuberosum*	Ku chai	>100
14	*Solanum melongena*	terong	>100
15	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	>100
16	*Cucumis sativus*	timun, timun China, mentimun	>100
17	*Momordica charantia*	peria	>100
18	*Luffa acutangula*	petola segi, ketola segi	>100
19	*Lactuca sativa*	salad, selada	>100
20	*Archidendron jiringga*	jering	>100
21	*Neptunia prostrata*	tangki,	>100

Table 4.5 continued

22	*Pachyrrhizus erosus*	sengkuwang,	>100
23	*Psophocarpus tetragonolobus*	kacang botor,	>100
24	*Parkia speciosa*	petai	>100
25	*Pisum sativum*	kacang puteh	>100
26	*Phaseolus vulgaris*	kacang buncis	>100
27	*Amaranthus gangeticus*	bayam merah	>100
28	*Amaranthus viridis*	bayam pasir	>100
29	*Hibiscus esculentus*	bendi	>100
30	*Averrhoa carambola*	belimbing besi	>100
31	*Manihot esculenta*	ubi kayu	>100
32	*Portulaca oleracea*	beremi	>100
33	*Ipomoea batatas*	keledek	>100
34	*Ipomoea reptans*	kangkung	>100
35	*Centella asiatica*	pegaga	>100
36	*Daucus carota*	lobak merah	>100
37	*Oenanthe javanica*	shelum	>100
38	*Beta vulgaris*	bit	>100
39	*Brassica oleracea* var. *alboglabra*	Kai lan	>100
40	*Petroselium crispum*	parsley	>100

4.2.3 *In vitro* Cytotoxic Activity of Selected Vegetable Extracts Against CaSki Cells

As illustrated in Figures 4.5-4.6, CaSki cells showed different cytotoxic profiles when treated with different crude extracts from selected vegetables. The *in vitro* growth inhibition of CaSki cells by crude methanol extracts of the 40 vegetables are shown as dose-response curves in Figure 4.5(a) to 4.5(h). The IC_{50} values were extrapolated from the dose-response curves and summarized in Table 4.6. The inhibition activities ranged from 1.39-44.70% at 1 µg/ml, 6.38-70.59% at 10 µg/ml, 14.62-88.94% at 25 µg/ml, 28.36-92.49% at 50 µg/ml, 39.89-93.95% at 100 µg/ml.

Out of 40 vegetables evaluated only 19 vegetables, namely *A. officinalis, A. fistulosum, S. melongena, S. tuberosum, Cucurbita moschata, L. sceraria, A. jiringga, N. prostrata, V. sinensis, P. sativum, P. vulgaris, A. gangeticus, A. viridis, H. esculentus, A. carambola, P. oleracea, I. reptans, B. vulgaris and B. oleracea* var. *alboglabra* were active against CaSki cells with IC_{50} values less than 20 µg/ml. The IC_{50} values ranged from 4.9 µg/ml to 19.8 µg/ml.

Crude methanol extracts of *Capsicum annum, Lycopersicum esculentum,Manihot esculenta* and *Petroselum crispum* inhibited CaSki cells with percentage of inhibition less than 50% at all concentrations tested in the present study. Therefore, IC_{50} values could not be determined from the existing dose-response curves.

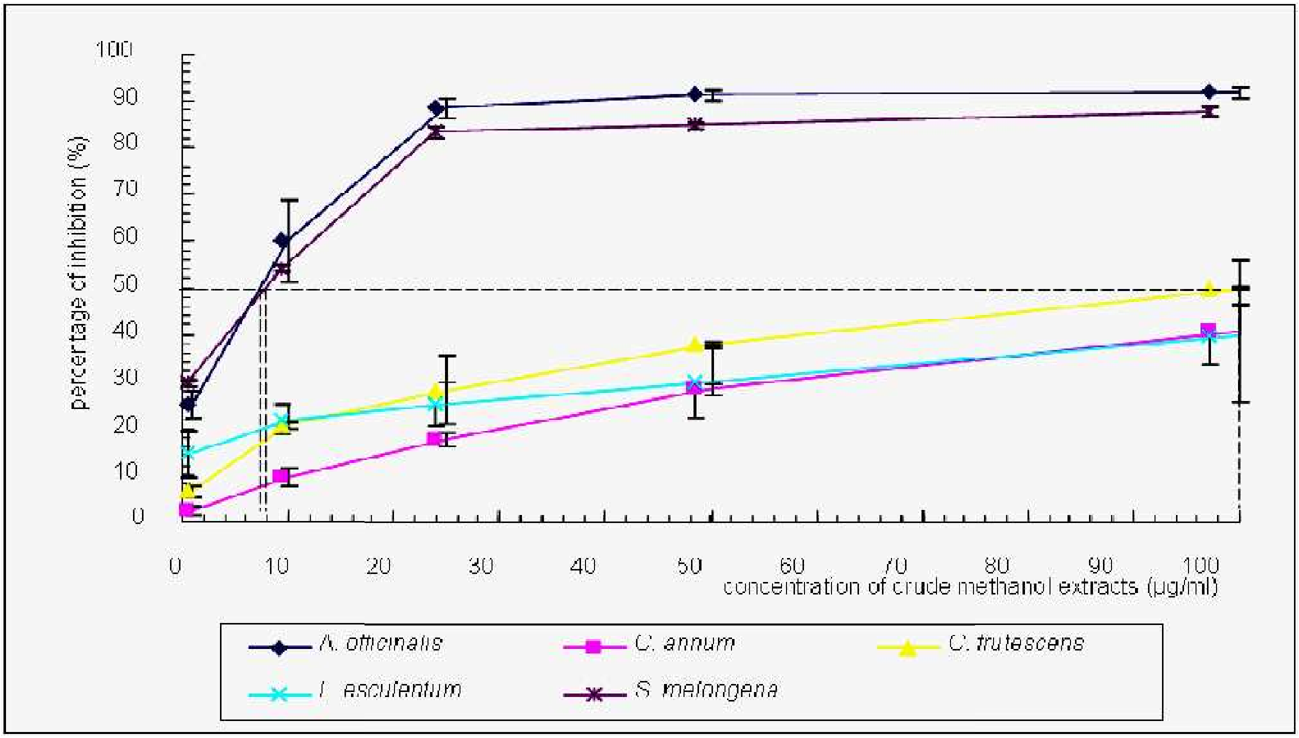


Figure 4.5 (a): Dose-response curves showing cytotoxic activity of crude methanol extract of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against CaSki cells

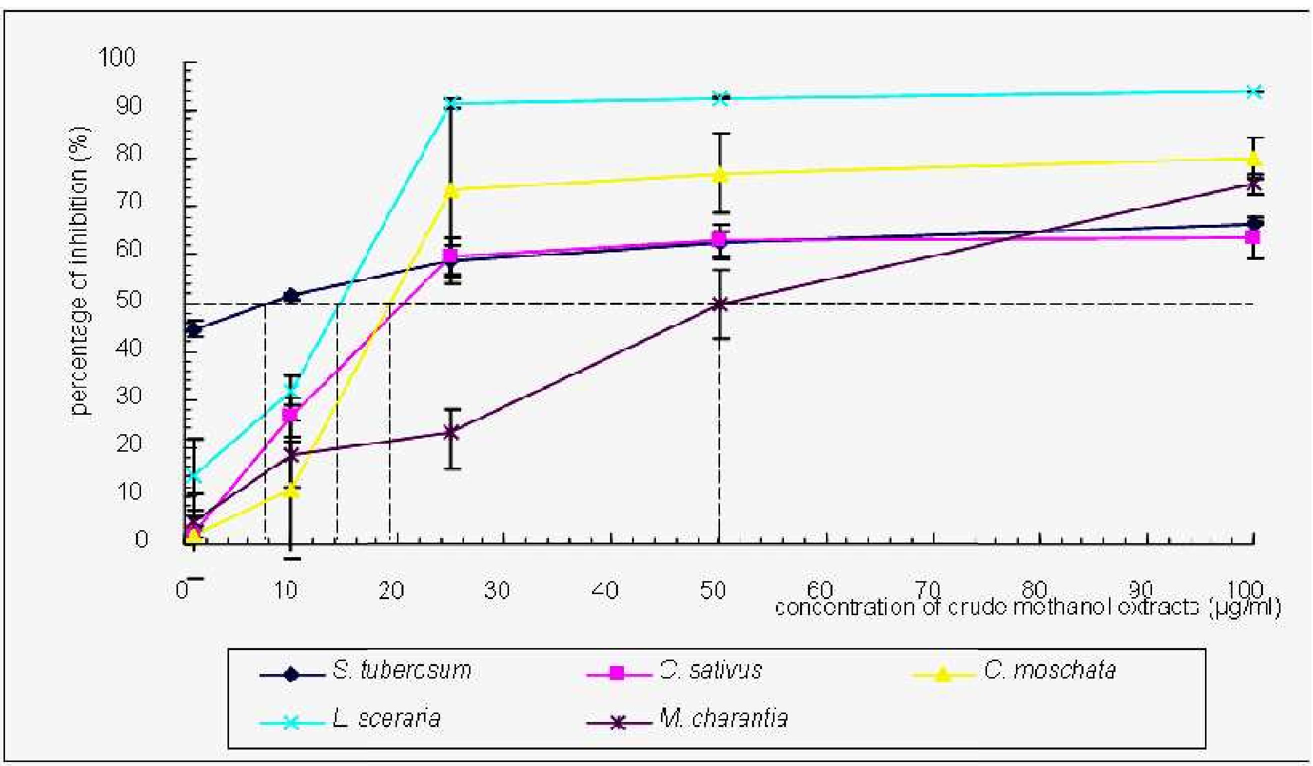


Figure 4.5 (b): Dose-response curves showing cytotoxic activity of crude methanol extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against CaSki cells

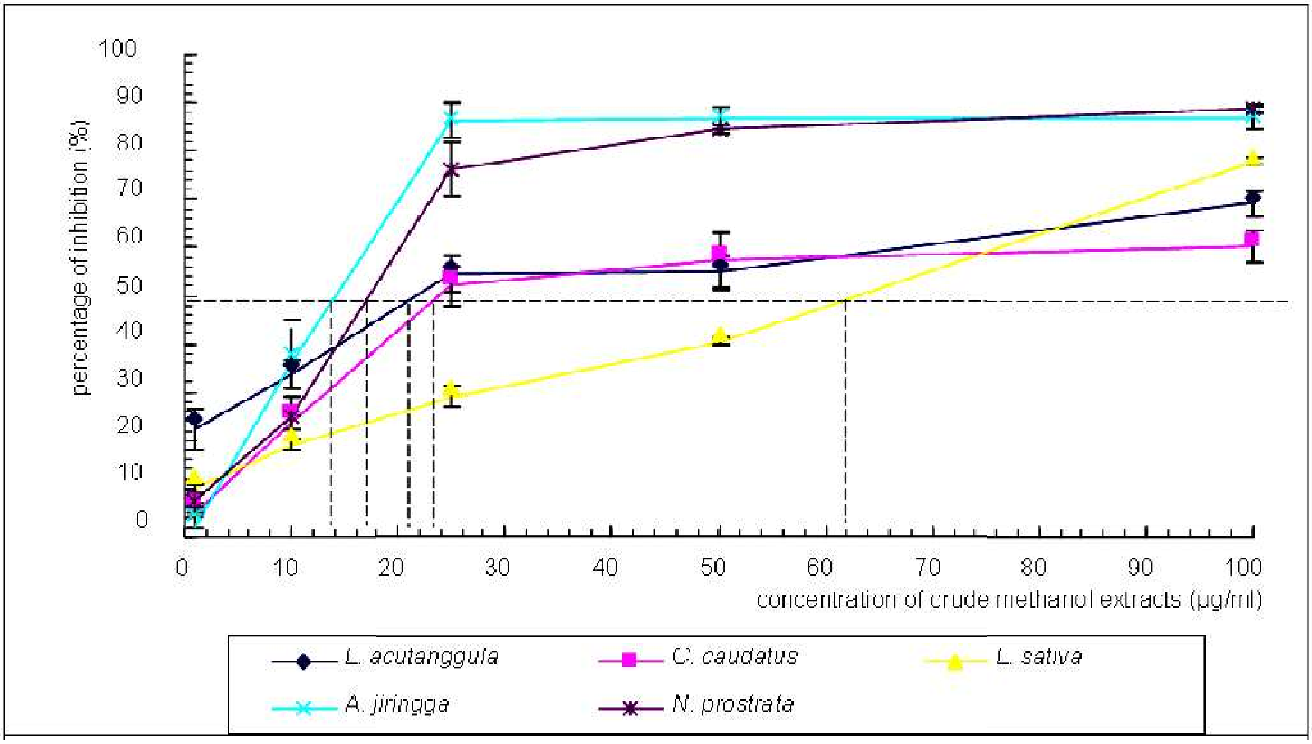


Figure 4.5 (c): Dose-response curves showing cytotoxic activity of crude methanol extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrata* against CaSki cells

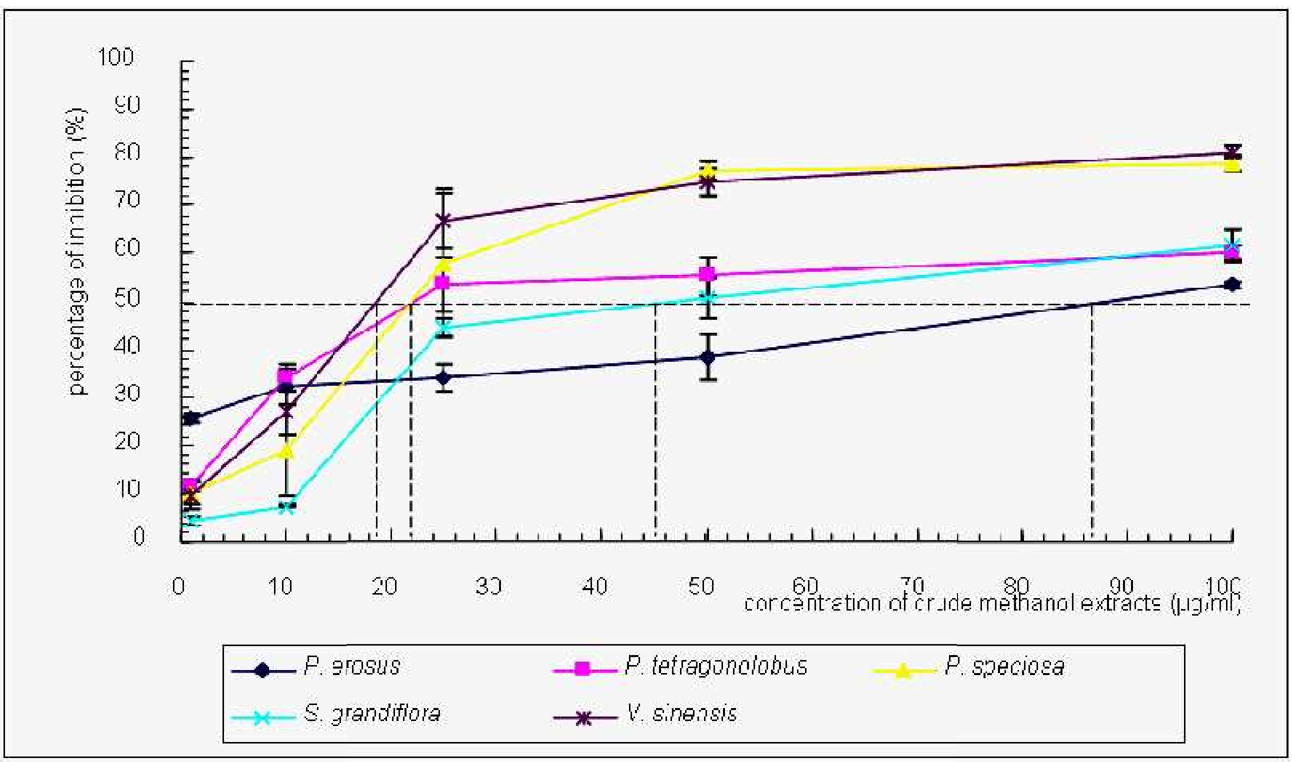


Figure 4.5 (d): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against CaSki cells

Figure 4.5 (e): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. gangeticus, A. viridis, H. esculentus, A. carambola* and *M. esculenta* against **CaSki cells**

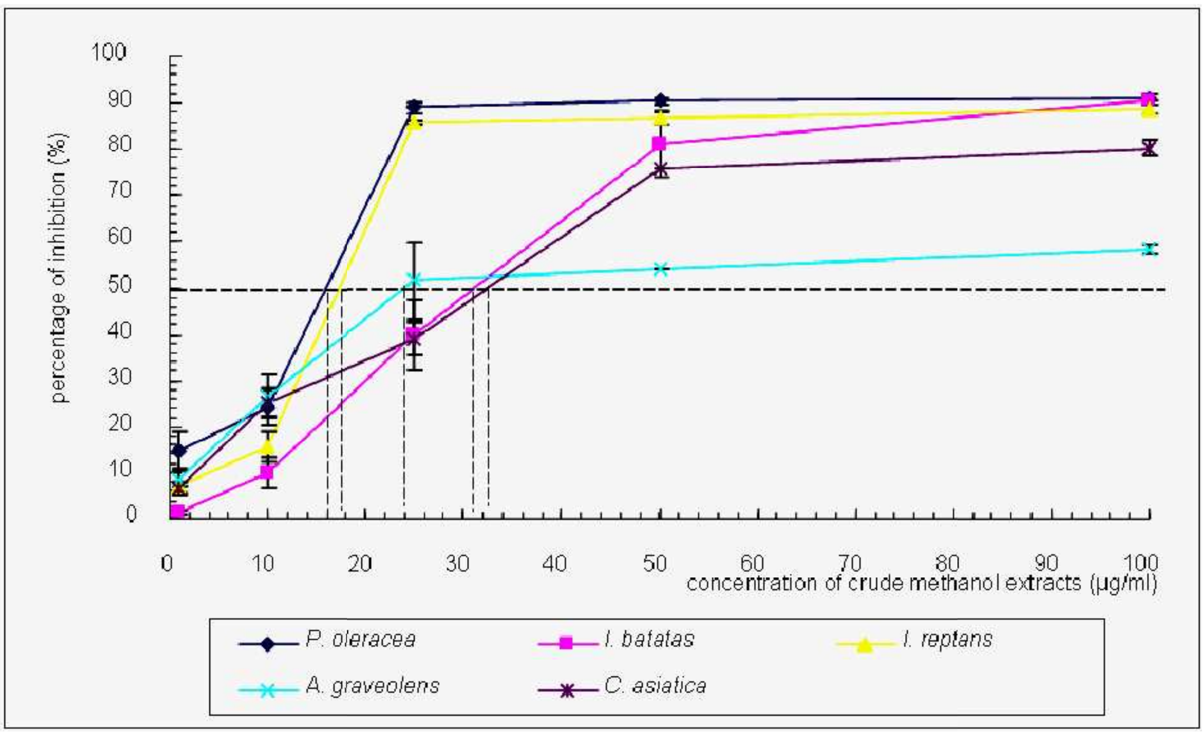
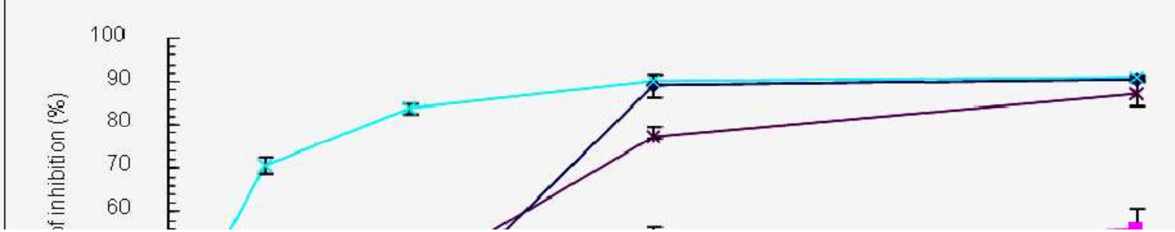


Figure 4.5 (f): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against CaSki cells



igure 4.5 (g): Dose-response curves showing cytotoxic activity of crude methanol extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against CaSki cells

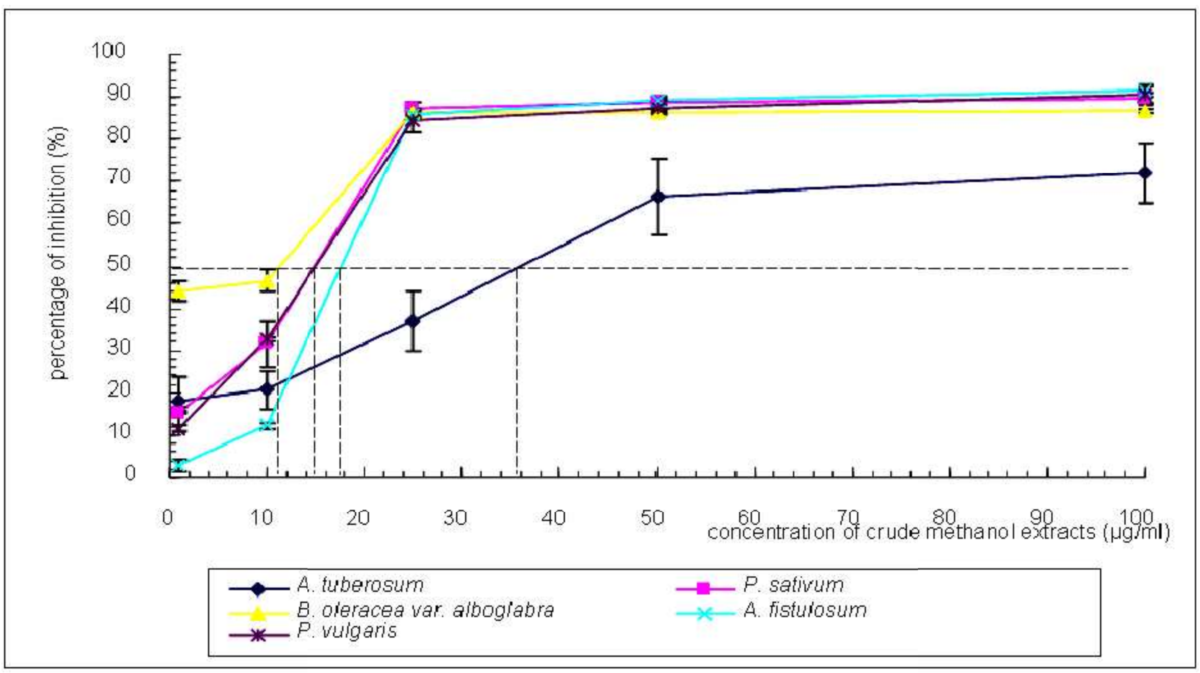


Figure 4.5 (h): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A.tuberosum, P. sativum, B. oleracea* var. *alboglabra, a. fistulosum,* and *P. vulgaris* against CaSki cells

Table 4.6: IC_{50} values of crude methanol of selected vegetables against CaSki cells

No	Plant	Local name	IC_{50} (µg/ml)
1	*Beta vulgaris*	bit	**4.90**
2	*Asparagus officinalis*	Asparagus, saparu keras	**7.80**
3	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	**7.80**
4	*Solanum melongena*	terong	**9.20**
5	*Brassica oleracea* var. *alboglabra*	Kai lan	**11.50**
6	*Archidendron jiringga*	jering	**13.90**
7	*Lagenaria sceraria*	labu ayer putih, labu botol	**14.10**
8	*Phaseolus vulgaris*	kacang buncis	**14.80**
9	*Pisum sativum*	kacang puteh	**14.80**
10	*Amaranthus gangeticus*	bayam merah	**16.00**
11	*Amaranthus viridis*	bayam pasir	**16.00**
12	*Portulaca oleracea*	beremi	**16.00**
13	*Hibiscus esculentus*	bendi	**17.00**
14	*Neptunia prostrata*	tangki,	**17.00**
15	*Allium fistulosum*	Daun bawang	**17.80**
16	*Ipomoea reptans*	kangkung	**17.80**
17	*Averrhoa carambola*	belimbing besi	**19.50**
18	*Cucurbita moschata*	labu merah, labu perang,	**19.80**
19	*Vigna sinensis*	kacang panjang	**19.80**
20	*Luffa acutangula*	petola segi, ketola segi	21.00
21	*Cucumis sativus*	timun, timun China, mentimun	22.00

Table 4.6 continued

22	*Parkia speciosa*	petai	22.00
23	*Psophocarpus tetragonolobus*	kacang botor,	22.00
24	*Cosmos caudatus*	ulam rajah	23.50
25	*Apium graveolens*	selderi	24.00
26	*Citrus aurantifolia*	limau	29.00
27	*Ipomoea batatas*	keledek	31.30
28	*Centella asiatica*	pegaga	32.50
29	*Daucus carota*	lobak merah	32.50
30	*Oenanthe javanica*	shelum	33.00
31	*Allium tuberosum*	Ku chai	35.80
32	*Sesbania grandiflora*	turi	45.00
33	*Momordica charantia*	peria	50.50
34	*Lactuca sativa*	salad, selada	62.00
35	*Pachyrrhizus erosus*	sengkuwang,	86.90
36	*Capsicum annum*	lada merah,	>100
37	*Capsicum frutescens*	chabai, lada api, lada kerawit	100
38	*Lycopersicum esculentum*	tomato	>100
39	*Manihot esculenta*	ubi kayu	>100
40	*Petroselium crispum*	parsley	>100

The *in vitro* growth inhibition of CaSki cells by crude water extracts of the 40 selected vegetables are shown as dose-response curves in Figures 4.6(a) to 4.6(h). The IC_{50} values were extrapolated from the dose-response curves and summarized in Table 4.7. The results reveal that the water extracts to be cytotoxic of CaSki cells with killing percentages of 4.57-41.36% at 1µg/ml, 9.7-65.66% at 10 µg/ml, 14.95-81.93% at 25 µg/ml, 23.65-84.92 at 50 µg/ml and 28.17-87.78 at 100 µg/ml.

Out of the 40 vegetables evaluated only 11 vegetables water extracts, namely *Asparagus officinalis*, *Capsicum annum, Lagenaria sceraria, Cosmos caudatus, Neptunia prostrata, psophocarpus tetragonolobus, Parkia speciosa, Vigna sinensis, phaseolus vulgaris, Hibiscus esculentus, Portulaca oleracea, and Beta vulgaris* were actively cytotoxic against CaSki cells with IC_{50} values less than 20 µg/ml. The IC_{50} values ranged were 5.9 µg/ml to 15 µg/ml.

Crude water extracts of *Pachyrrhizus erosus*, *Amaranthus gangeticus, Solanum melongena, Averrhoa carambola, Manihot esculenta, Apium graveolens, Oenanthe javanica, and Citrus aurantifolia* inhibited CaSki cells with percentage of inhibition less than 50% at all concentrations tested in this study. Therefore, IC_{50} values could not be determined from the existing dose-response curves.

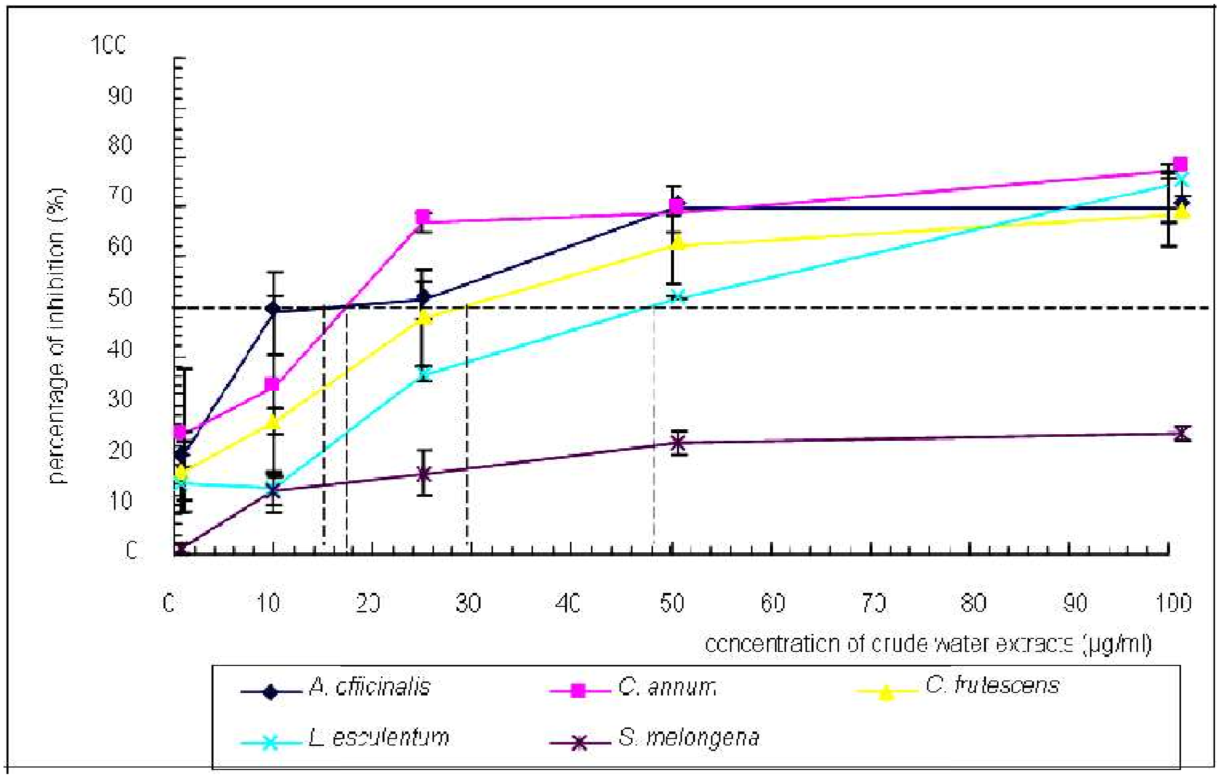


Figure 4.6 (a): Dose-response curves showing cytotoxic activity of crude water extracts of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against CaSki cells

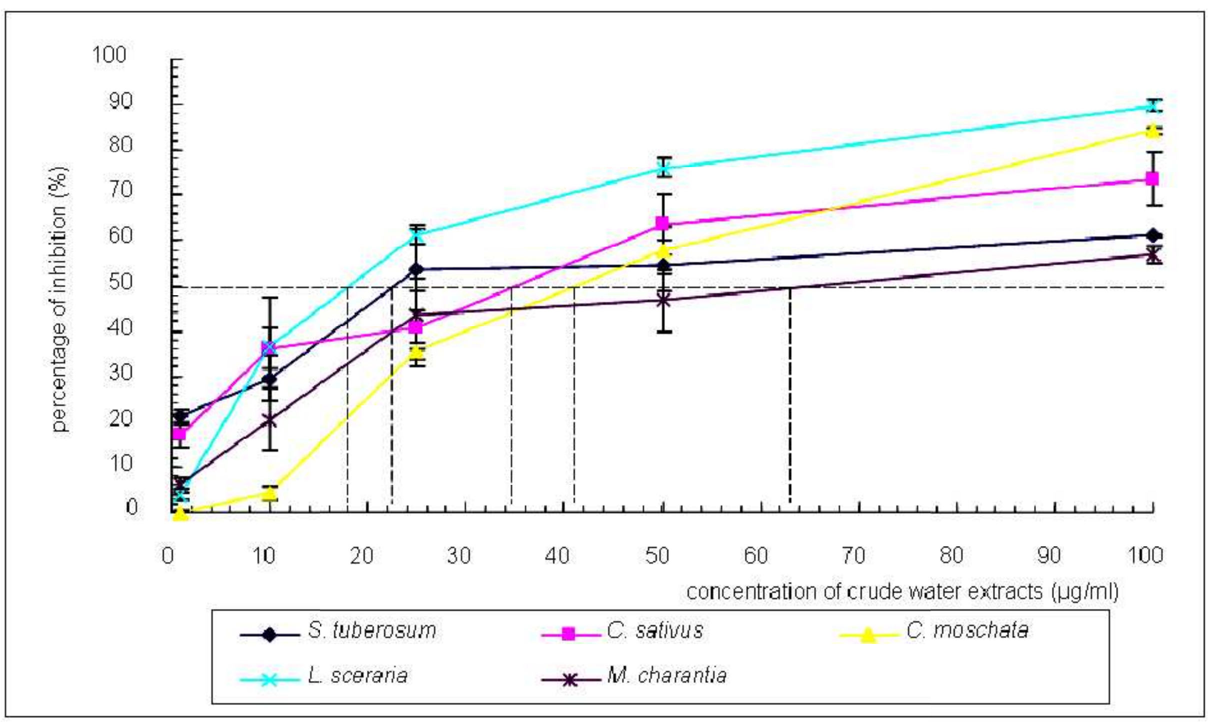


Figure 4.6 (b): Dose-response curves showing cytotoxic activity of crude water extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against CaSki cells

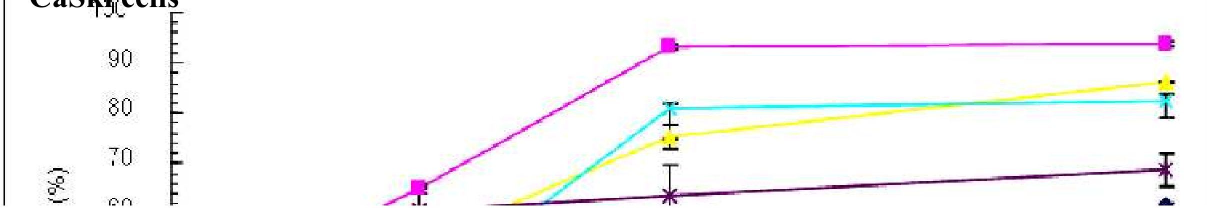


Figure 4.6 (c): Dose-response curves showing cytotoxic activity of crude water extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrata* against CaSki cells

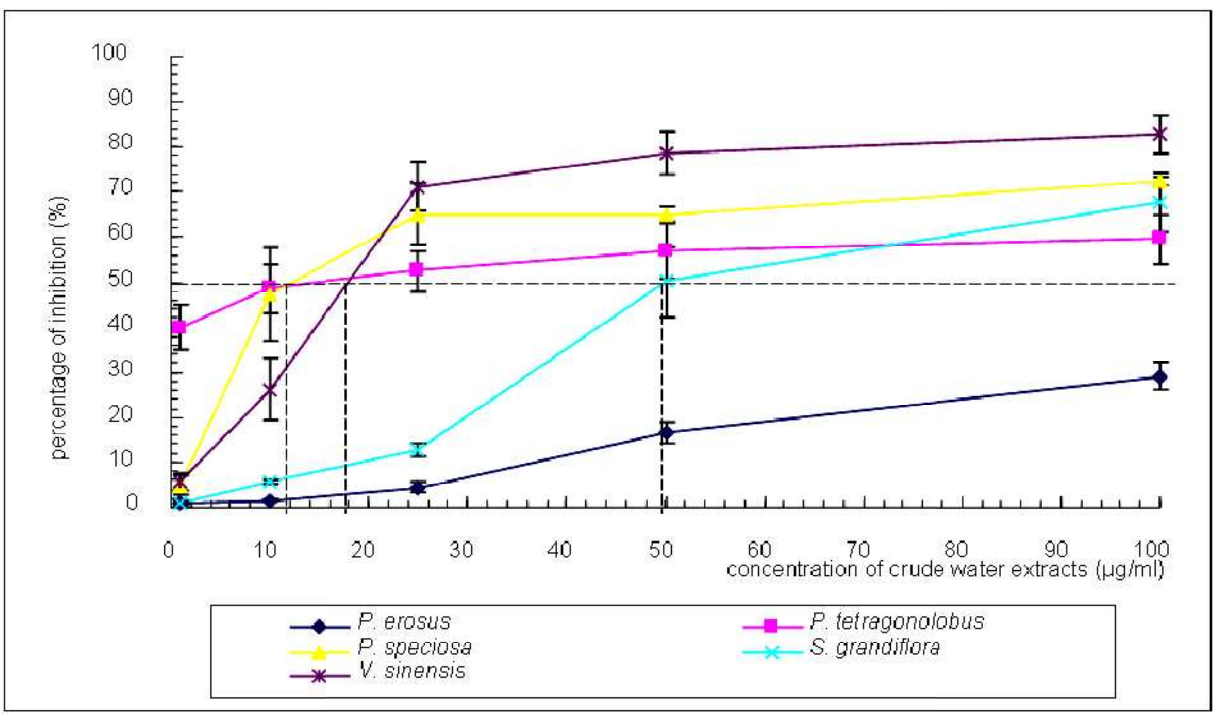


Figure 4.6 (d): Dose-response curves showing cytotoxic activity of crude water extracts of *P.erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against CaSki cells

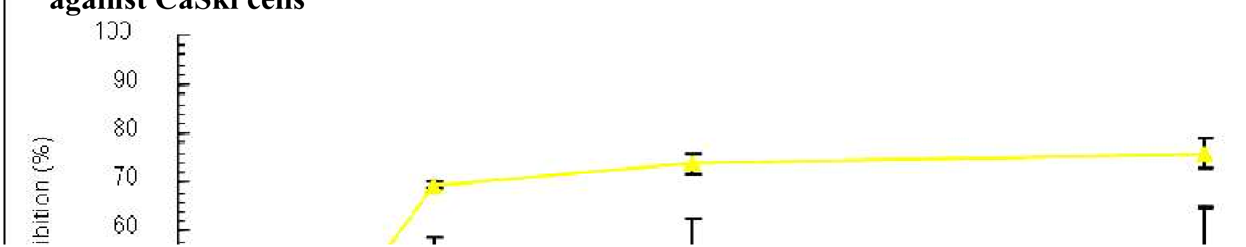


Figure 4.6 (e): Dose-response curves showing cytotoxic activity of crude water extracts of *A. gangeticus, A. viridis, H. esculentus, A. carambola* and *M. esculenta* against CaSki cells

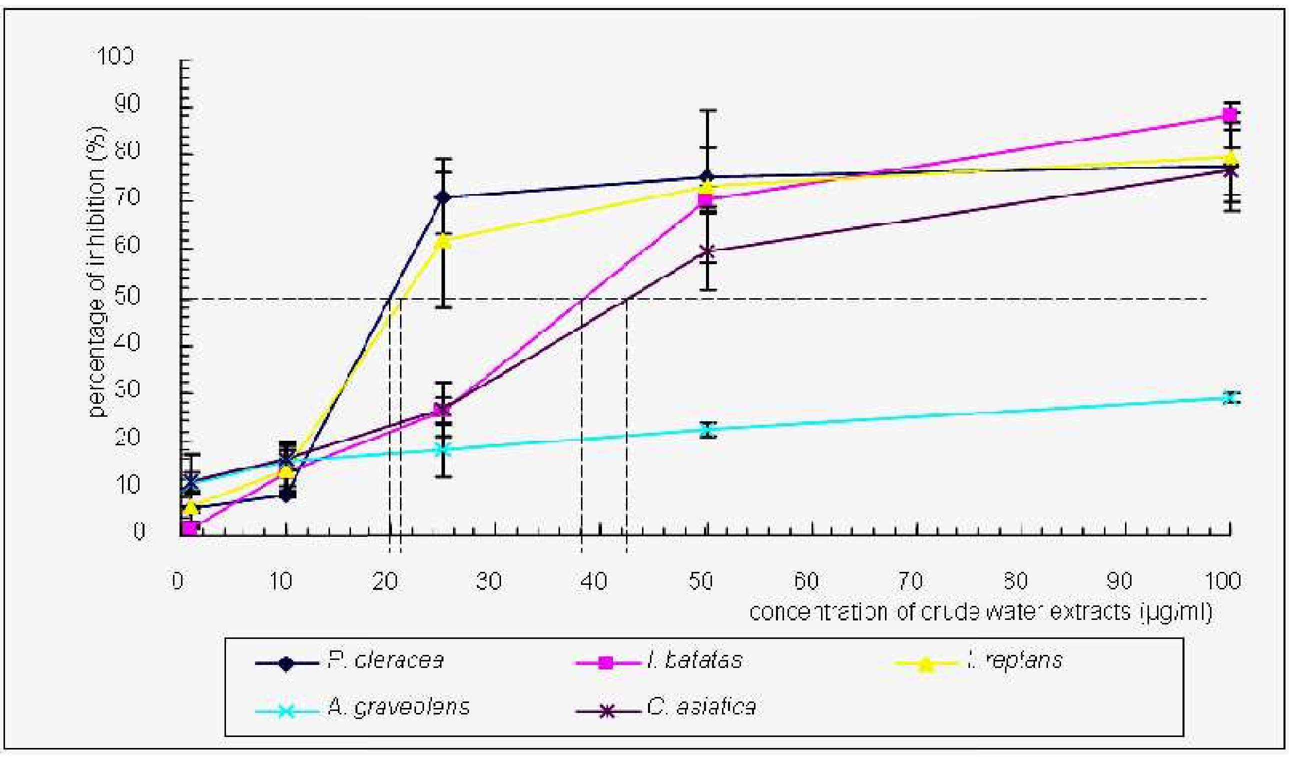


Figure 4.6 (f): Dose-response curves showing cytotoxic activity of crude water extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against CaSki cells

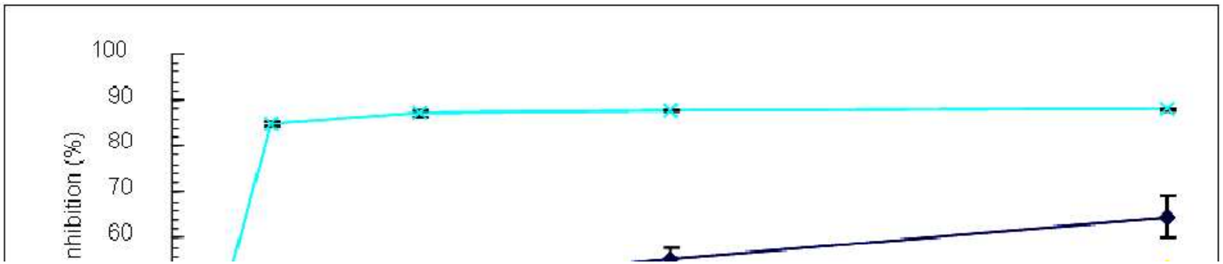


Figure 4.6 (g): Dose-response curves showing cytotoxic activity of crude water extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against CaSki cells

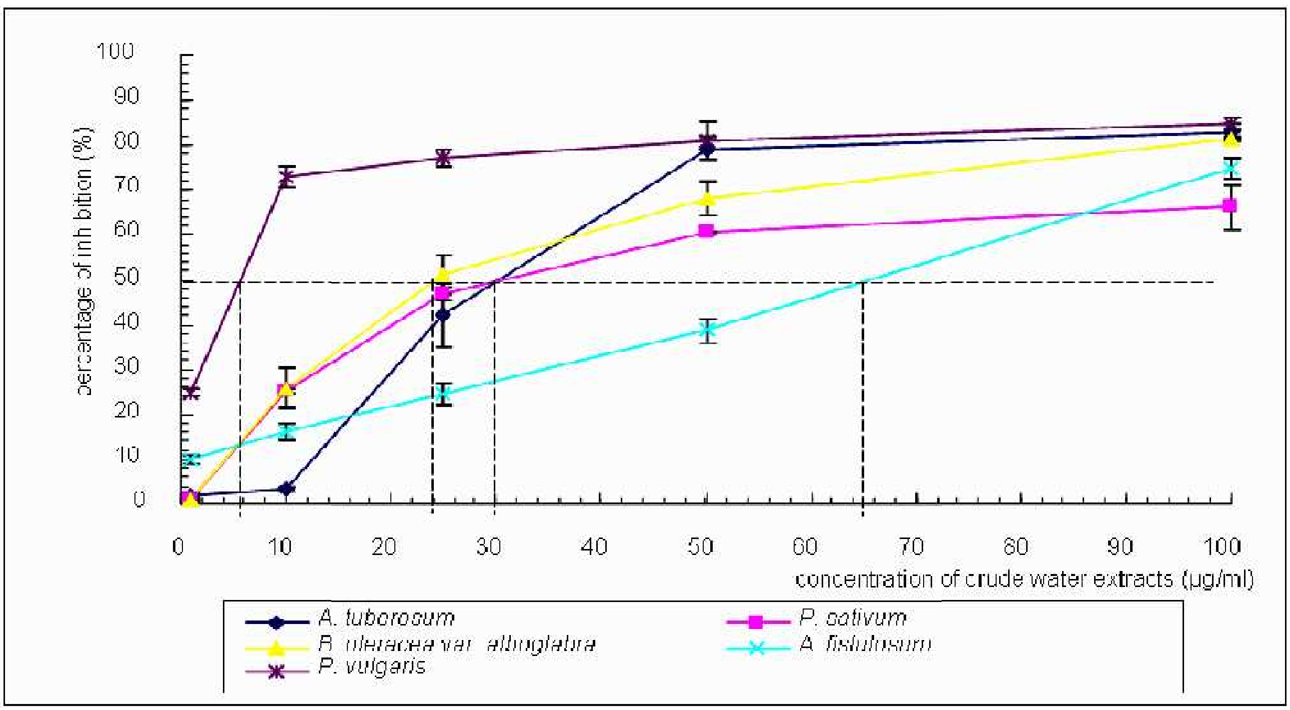


Figure 4.6 (h): Dose-response curves showing cytotoxic activity of crude water extracts of *A.tuberosum, P. sativum, B. oleracea* var. *alboglabra, a. fistulosum* against CaSki cells

Table 4.7:IC_{50} values of crude water extracts of selected vegetables against CaSki cells

No	**Plant**	**Local name**	IC_{50} (µg/ml)
1	*Beta vulgaris*	bit	**5.90**
2	*Phaseolus vulgaris*	kacang buncis	**5.90**
3	*Cosmos caudatus*	ulam rajah	**10.00**
4	*Psophocarpus tetragonolobus*	kacang botor,	**10.00**
5	*Parkia speciosa*	petai	**11.50**
6	*Asparagus officinalis*	Asparagus, saparu keras	**15.00**
7	*Capsicum annum*	lada merah,	**17.50**
8	*Hibiscus esculentus*	bendi	**18.00**
9	*Lagenaria sceraria*	labu ayer putih, labu botol	**18.00**
10	*Vigna sinensis*	kacang panjang	**18.00**
11	*Neptunia prostrata*	tangki,	**18.20**
12	*Portulaca oleracea*	beremi	**20.00**
13	*Ipomoea reptans*	kangkung	22.00
14	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	22.50
15	*Daucus carota*	lobak merah	23.00
16	*Lactuca sativa*	salad, selada	23.50
17	*Brassica oleracea* var. *alboglabra*	Kai lan	24.00
18	*Archidendron jiringga*	jering	29.00
19	*Capsicum frutescens*	chabai, lada api, lada kerawit	29.50
20	*Allium tuberosum*	Ku chai	30.00
21	*Pisum sativum*	kacang puteh	30.00

Table 4.7 continued

22	*Amaranthus viridis*	bayam pasir	33.50
23	*Cucumis sativus*	timun, timun China, mentimun	34.50
24	*Ipomoea batatas*	keledek	38.00
25	*Cucurbita moschata*	labu merah, labu perang,	41.50
26	*Centella asiatica*	pegaga	42.50
27	*Lycopersicum esculentum*	tomato	48.50
28	*Sesbania grandiflora*	turi	49.50
29	*Luffa acutangula*	petola segi, ketola segi	63.00
30	*Momordica charantia*	peria	63.00
31	*Allium fistulosum*	Daun bawang	65.00
32	*Petroselium crispum*	parsley	79.00
33	*Amaranthus gangeticus*	bayam merah	>100
34	*Apium graveolens*	selderi	>100
35	*Averrhoa carambola*	belimbing besi	>100
36	*Citrus aurantifolia*	limau	>100
37	*Manihot esculenta*	ubi kayu	>100
38	*Oenanthe javanica*	shelum	>100
39	*Pachyrrhizus erosus*	sengkuwang,	>100
40	*Solanum melongena*	terong	>100

4.2.4 *In vitro* Cytotoxic Activity of Crude Methanol and Water Extracts of Selected Vegetable Against MRC5 Cells

The *in vitro* cytotoxicity activities of crude methanol and water extracts against MRC5 cells are illustrated as dose-response curves in Figures 4.7(a-h) and 4.8(a-h). As before the IC_{50} values for all crude extracts were extrapolated from the dose-response curves and summarized in Tables 4.8 and 4.9.

Generally, all crude methanol extracts from the 40 selected vegetables demonstrated no cytotoxic activities against MRC5 cells. The killing percentages produced by the methanol extracts ranged from 2.74-32.33 at 1 µg/ml, 8.2-40.20% at 10 µg/ml, 12.46-44.53% at 25 µg/ml, 20.39-50.35% at 50 µg/ml, 25.00-52.98% at 100 µg/ml. Only 8 out of 40 selected vegetable extracts, have IC_{50} values which could be extrapolated from dose-respone curves. The IC_{50} values extrapolated were between 48 µg/ml to 96 µg/ml produced by *Asparagus officinalis*, *Allium tuberosum, A. Centella asiatica*, *Vigna sinensis*, *Capsicum annum*, *Psophocarpus tetragonolobus*, *Solanum melongena* and *Citrus aurantifolia* (Table 4.8). The IC_{50} values for the other vegetable extracts could not be determined from the existing killing-curves.

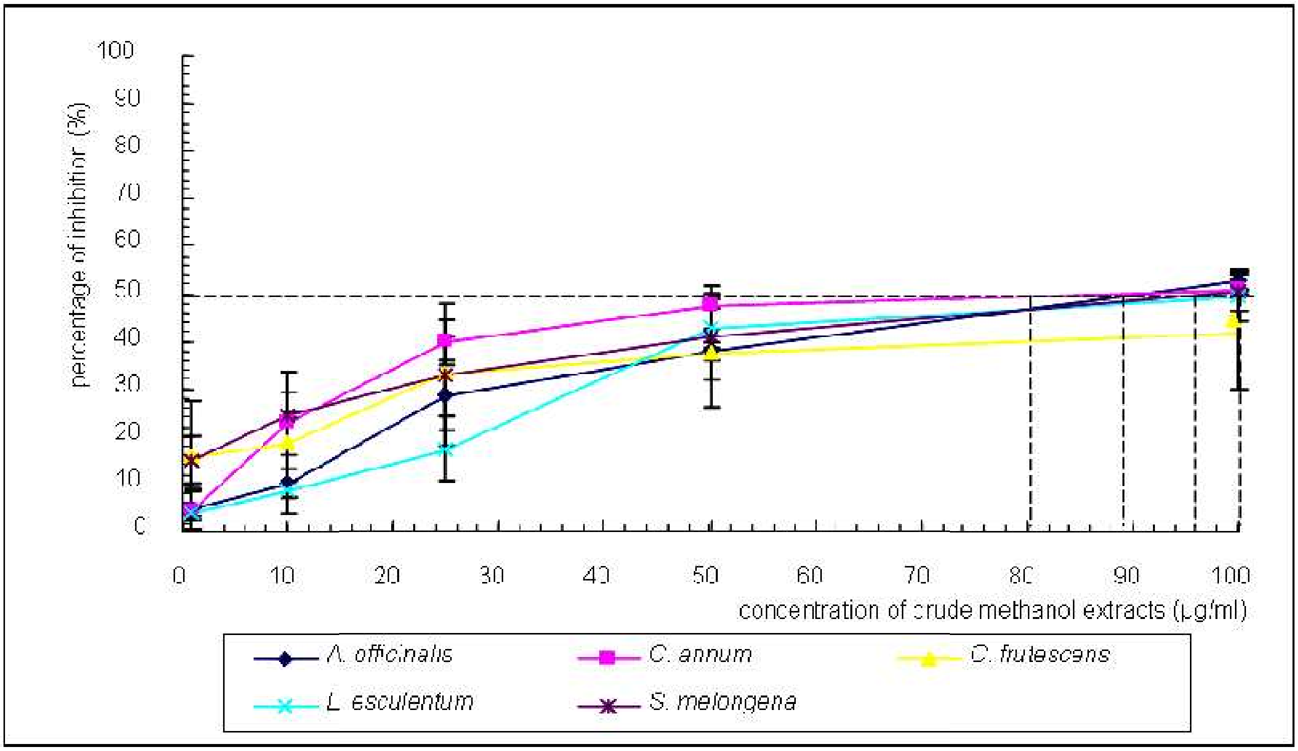


Figure 4.7 (a): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against MRC5 cells

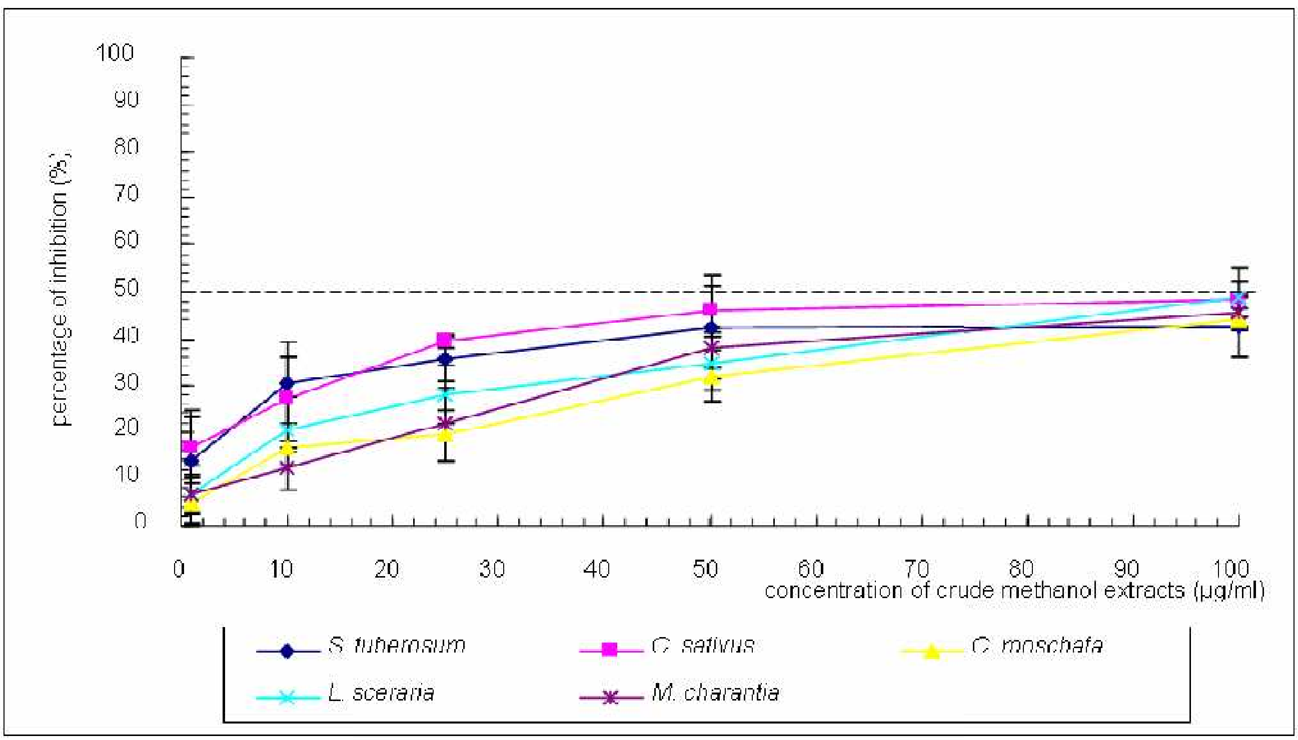


Figure 4.7 (b): Dose-response curves showing cytotoxic activity of crude methanol extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against MRC5 cells

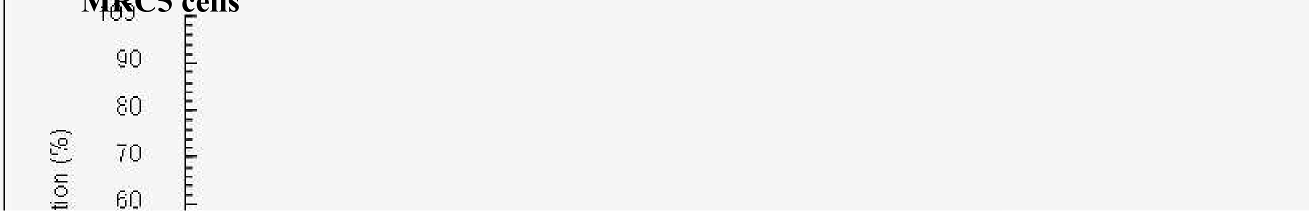


Figure 4.7 (c): Dose-response curves showing cytotoxic activity of crude methanol extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrata* against MRC5 cells

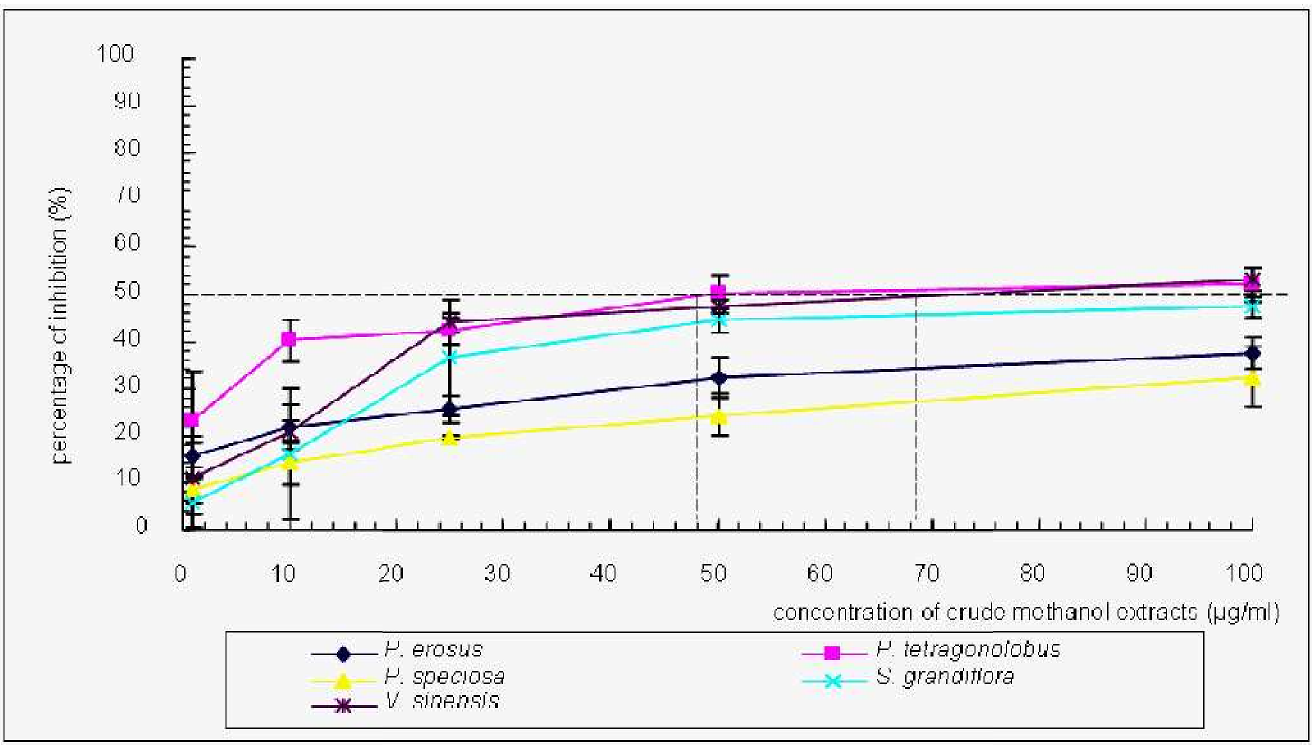


Figure 4.7 (d): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against MRC5 cells

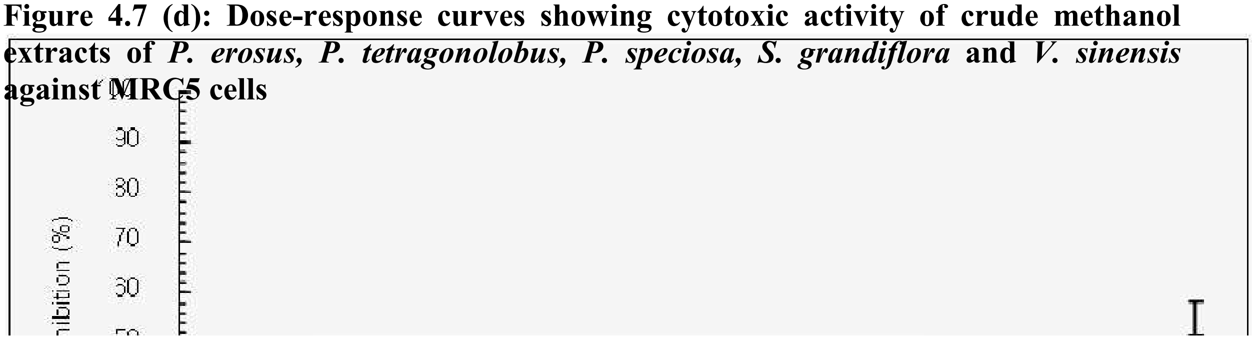


Figure 4.7 (e): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. gangeticus, A. viridis, H. esculentus, A. carambola* and *M. esculenta* against MRC5 cells

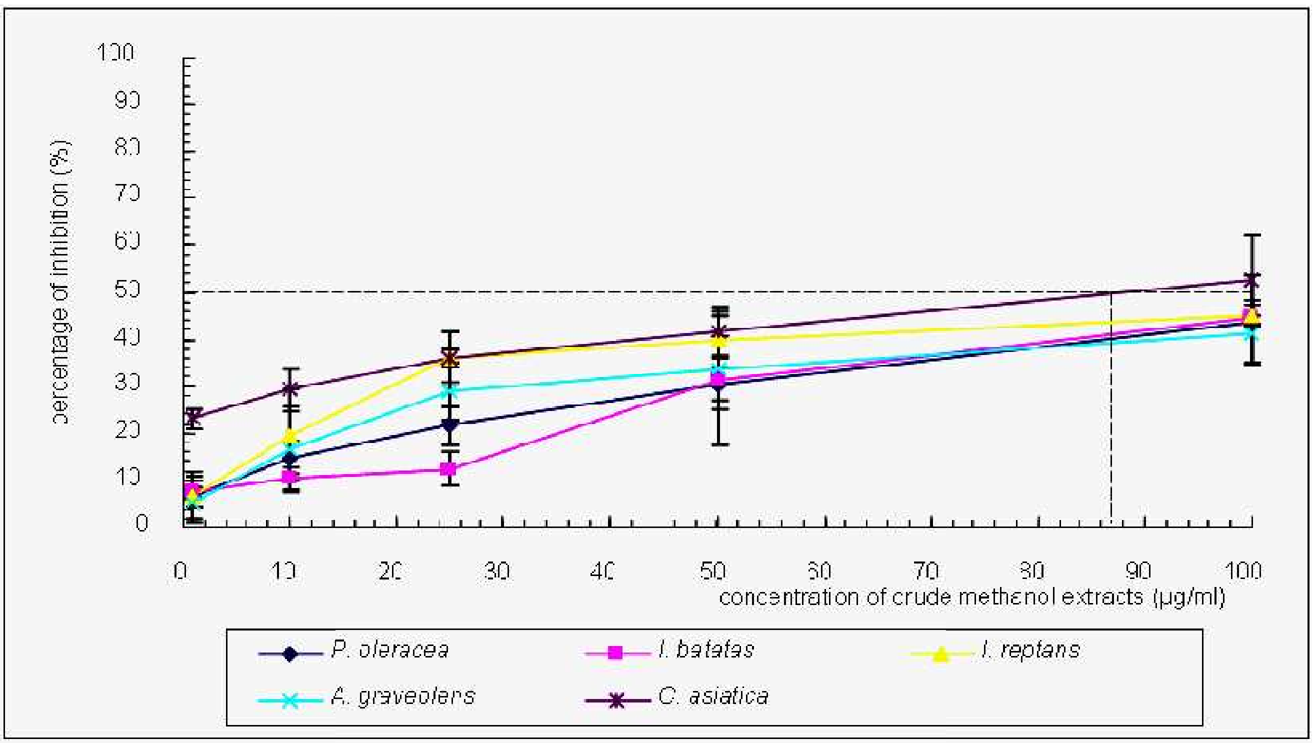


Figure 4.7 (f): Dose-response curves showing cytotoxic activity of crude methanol extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against MRC5 cells

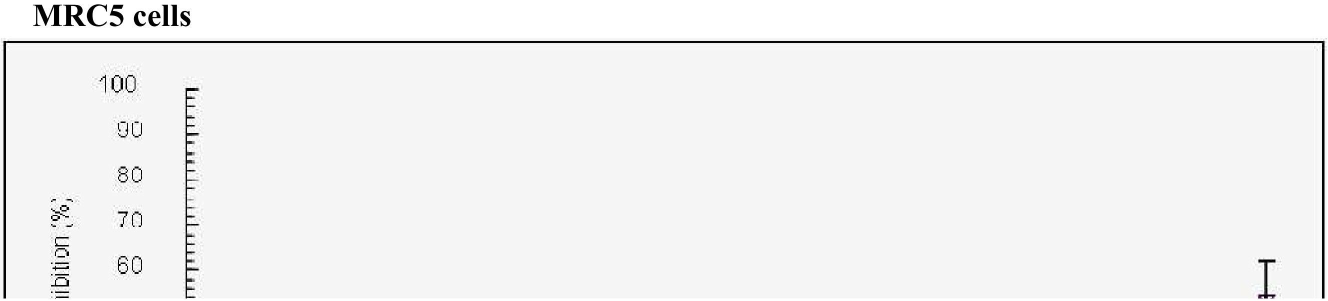


Figure 4.7 (g): Dose-response curves showing cytotoxic activity of crude methanol extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against MRC5 cells

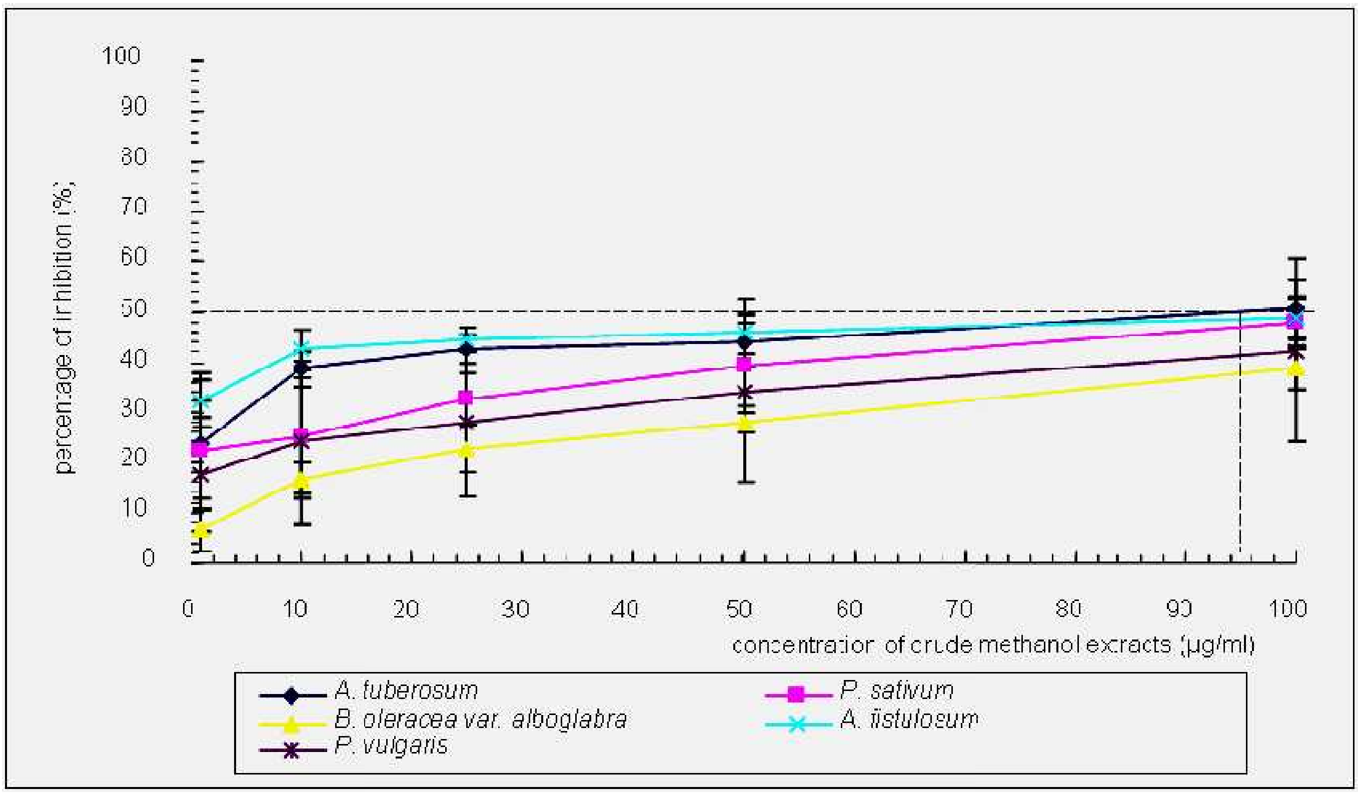


Figure 4.7 (h): Dose-response curves showing cytotoxic activity of crude methanol extracts of *A. tuberosum, P. sativum, B. oleracea var. alboglabra* and *P. vulgaris* against MRC5 cells

Table 4.8: IC_{50} values of crude methanol extracts of selected vegetables against MRC5 cells

No	Plant	Local name	IC_{50} (µg/ml)
1	*Psophocarpus tetragonolobus*	kacang botor	48.00
2	*Vigna sinensis*	kacang panjang	68.500
3	*Citrus aurantifolia*	limau	77.80
4	*Capsicum annum*	lada merah	80.00
5	*Centella asiatica*	Pegaga	87.00
6	*Asparagus officinalis*	Asparagus, saparu keras	89.00
7	*Allium tuberosum*	Ku chai	95.00
8	*Solanum melongena*	terong	96.00
9	*Allium fistulosum*	Daun bawang	>100
10	*Amaranthus gangeticus*	bayam merah	>100
11	*Amaranthus viridis*	bayam pasir	>100
12	*Apium graveolens*	Selderi	>100
13	*Archidendron jiringga*	jering	>100
14	*Averrhoa carambola*	belimbing besi	>100
15	*Beta vulgaris*	Bit	>100
16	*Brassica oleracea* var. *alboglabra*	Kai lan	>100
17	*Capsicum frutescens*	chabai, lada api, lada kerawit	>100
18	*Cosmos caudatus*	ulam rajah	>100
19	*Cucumis sativus*	timun, timun China, mentimun	>100
20	*Cucurbita moschata*	labu merah, labu perang,	>100
21	*Daucus carota*	lobak merah	>100

Table 4.8 continued

22	*Hibiscus esculentus*	Bendi	>100
23	*Ipomoea batatas*	keledek	>100
24	*Ipomoea reptans*	kangkung	>100
25	*Lactuca sativa*	salad, selada	>100
26	*Lagenaria sceraria*	labu ayer putih, labu botol	>100
27	*Luffa acutangula*	petola segi, ketola segi	>100
28	*Lycopersicum esculentum*	tomato	>100
29	*Manihot esculenta*	ubi kayu	>100
30	*Momordica charantia*	peria	>100
31	*Neptunia prostrata*	tangki	>100
32	*Oenanthe javanica*	shelum	>100
33	*Pachyrrhizus erosus*	sengkuwang	>100
34	*Parkia speciosa*	petai	>100
35	*Petroselium crispum*	parsley	>100
36	*Phaseolus vulgaris*	kacang buncis	>100
37	*Pisum sativum*	kacang puteh	>100
38	*Portulaca oleracea*	beremi	>100
39	*Sesbania grandiflora*	turi	>100
40	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	>100

The *in vitro* cytotoxicity activities of crude water extracts against MRC 5 cells are illustrated as dose-response curves in Figures 4.8(a)-4.8(h). The IC_{50} values were extrapolated from the dose-response curves and summarized in Table 4.9. Only 7 out of 40 selected vegetable extracts, have IC_{50} values which could be extrapolated from dose-respone curves. The IC_{50} values extrapolated which were between 59.5 µg/ml-97.5 µg/ml was produced by *Allium tuberosum*, *A. fistulosum, Centella asiatica*, *Vigna sinensis*, *Luffa acutanggula, Hibiscus esculentus, Averrhoa carambola, Apium graveolen and Citrus aurantifolia*. The IC_{50} value for the other vegetable extracts could not be determined from the existing killing-curves.

Overall results indicated that, the crude methanol and water extracts from the 40 selected vegetables were found to be not cytotoxic against MRC5 cell lines.

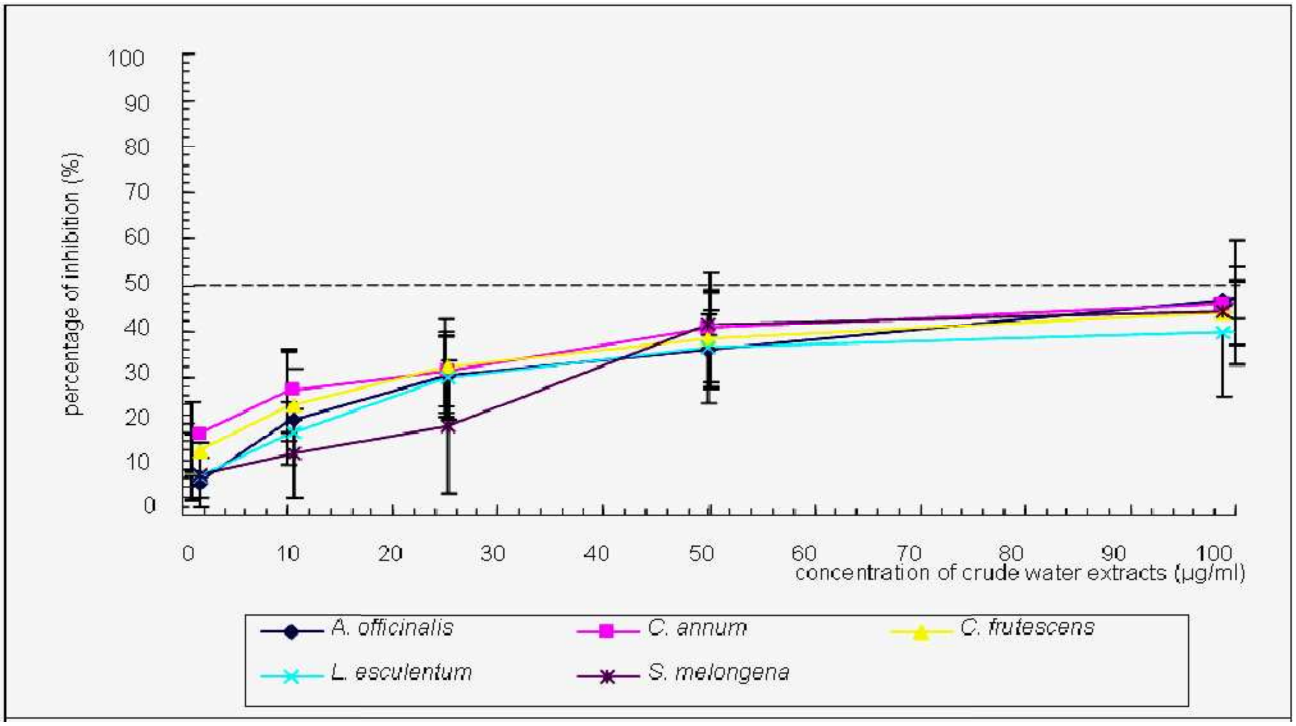


Figure 4.8 (a): Dose-response curves showing cytotoxic activity of crude water extracts of *A. officinalis*, *C. annum*, *C. frutescens*, *L. esculentum* and *S. melongena* against MRC5 cells

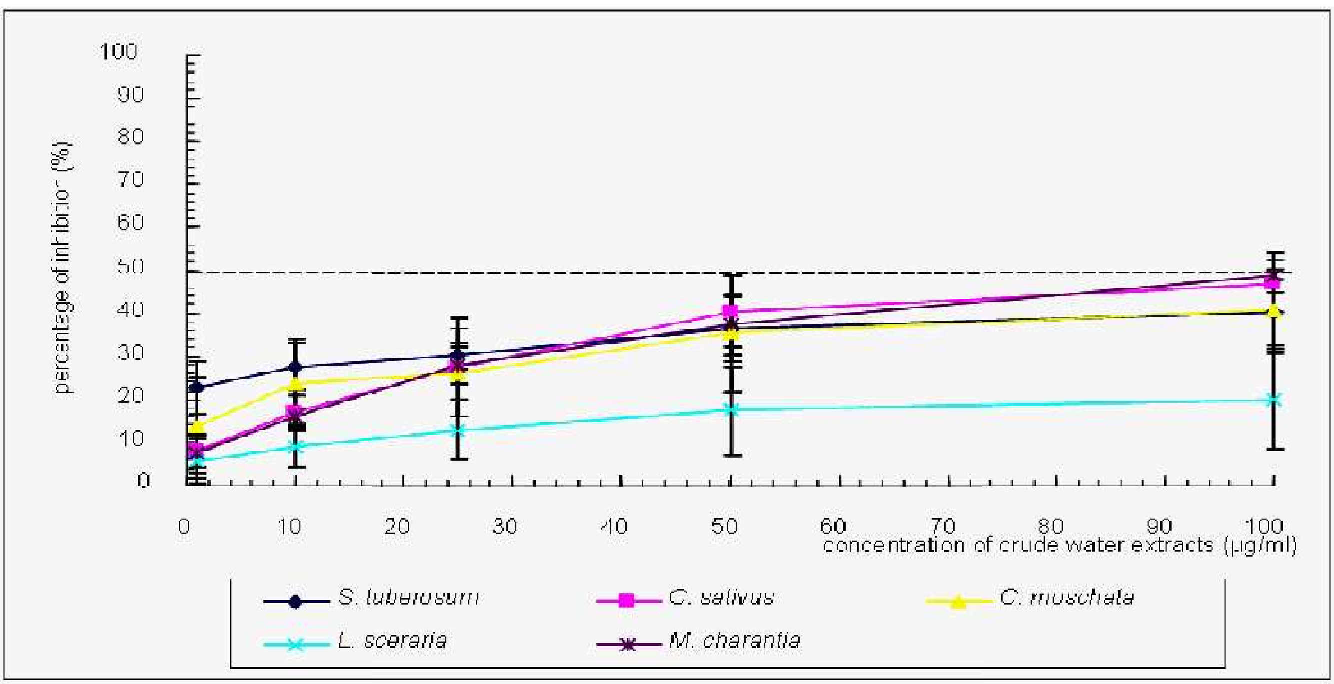


Figure 4.8 (b): Dose-response curves showing cytotoxic activity of crude water extracts of *S. tuberosum, C. sativus, C. moschata, L. sceraria* and *M. charantia* against MRC5 cells

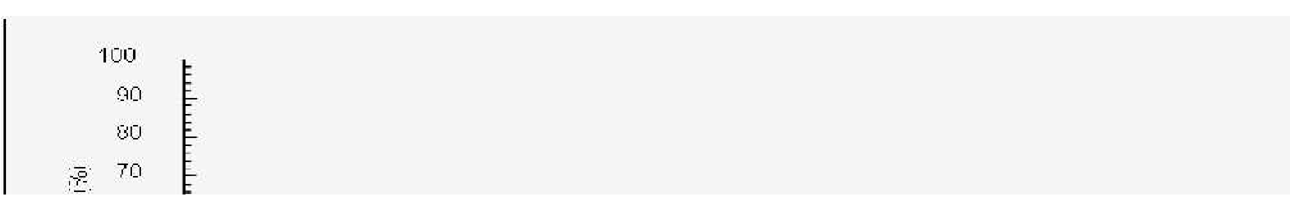


Figure 4.8 (c): Dose-response curves showing cytotoxic activity of crude water extracts of *L. acutanggula, C. caudatus, L. sativa, A. jiringga* and *N. prostrata* against MRC5 cells

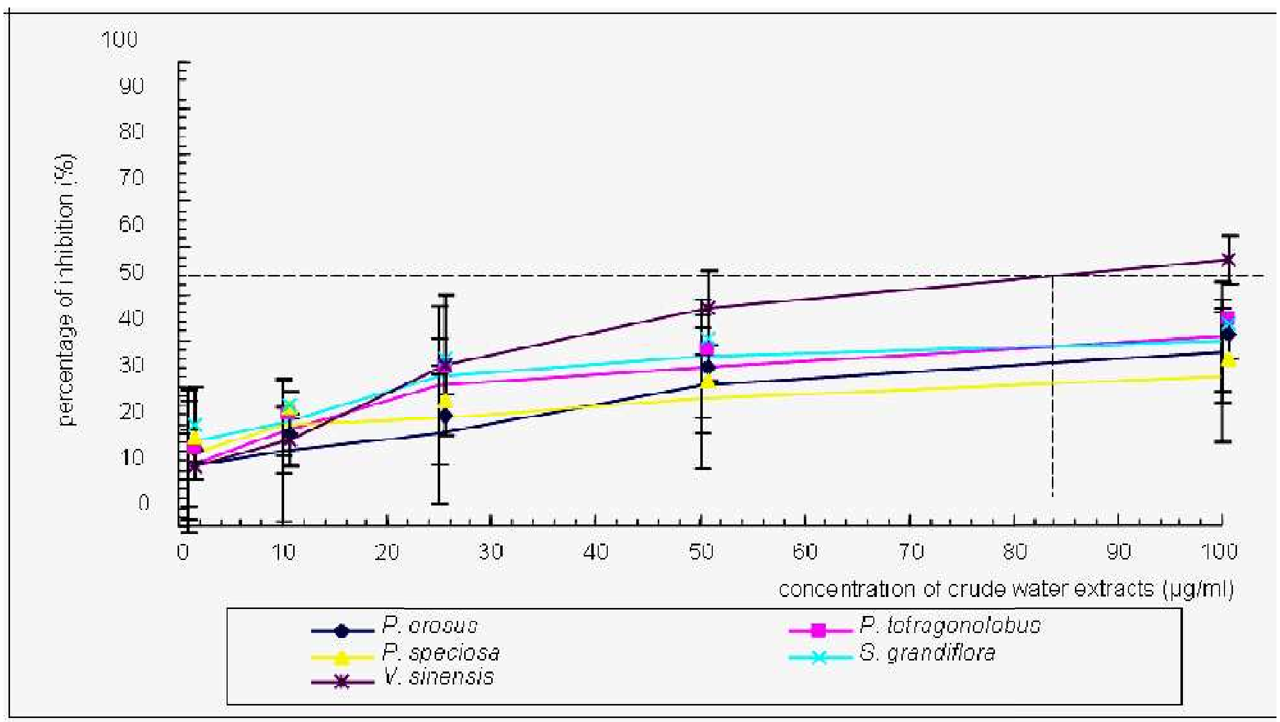


Figure 4.8 (d): Dose-response curves showing cytotoxic activity of crude water extracts of *P.erosus, P. tetragonolobus, P. speciosa, S. grandiflora* and *V. sinensis* against MRC5 cells

Figure 4.8 (e): Dose-response curves showing cytotoxic activity of crude water extracts of *A. gangeticus, A. viridis, H. esculentus, A. carambola* and *M. esculenta* against MRC5 cells

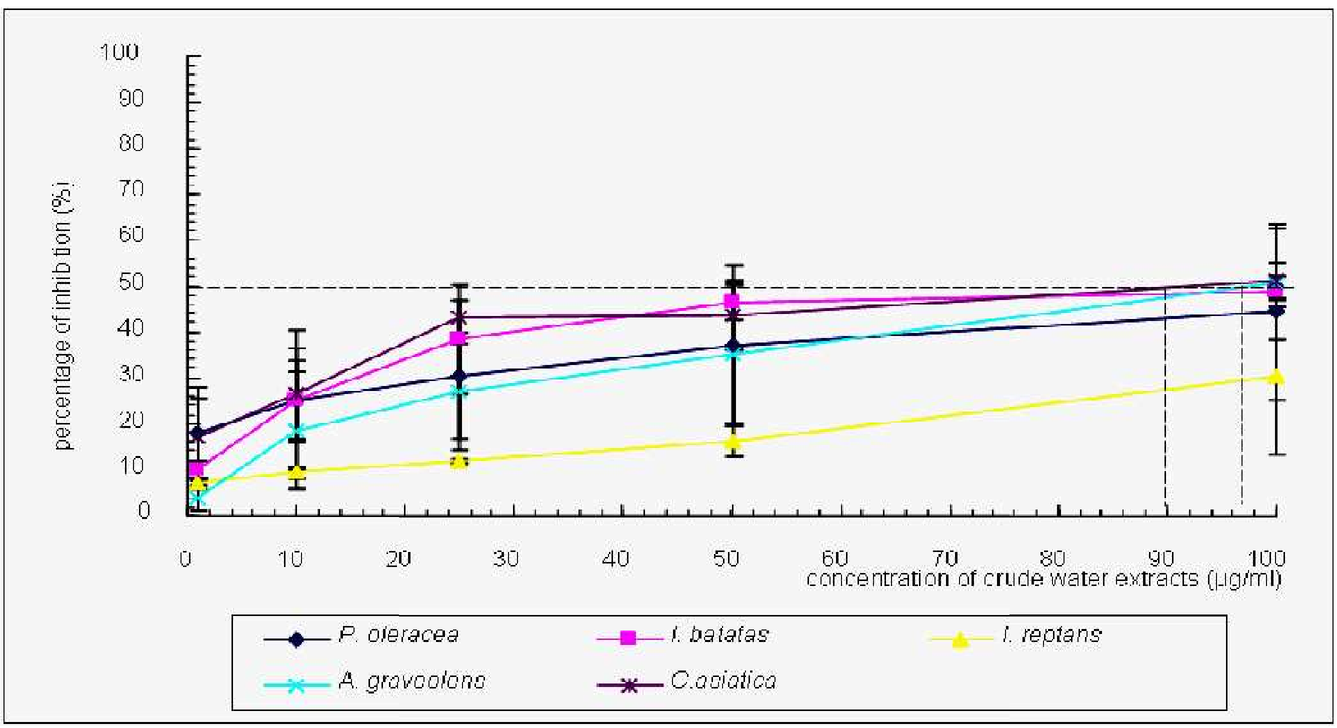


Figure 4.8 (f): Dose-response curves showing cytotoxic activity of crude water extracts of *P. oleracea, I. batatas, I. reptans, A. graveolens* and *C. asiatica* against MRC5 cells

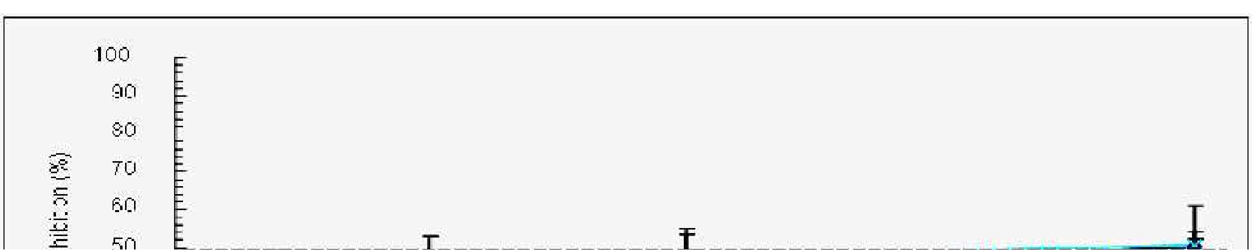


Figure 4.8 (g): Dose-response curves showing cytotoxic activity of crude water extracts of *D. carota, O. javanica, P. crispum, B. vulgaris* and *C. aurantifolia* against MRC5 cell

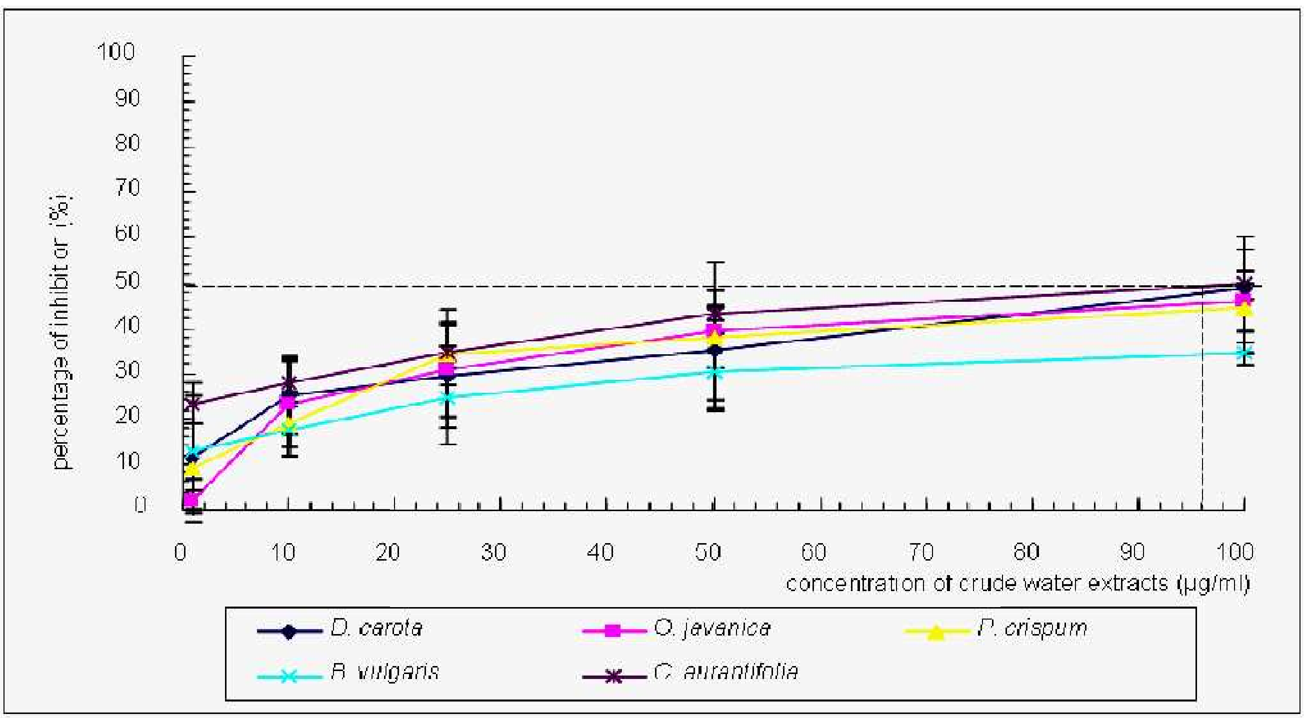


Figure 4.8 (h): Dose-response curves showing cytotoxic activity of crude water extracts of *A.tuberosum, P. sativum, B. oleracea* var. *alboglabra, A. fistulosum,* and *P. vulgaris* against MRC5 c

Table 4.9: IC_{50} values of crude water extracts of selected vegetables against MRC5 cells

No	Plant	Local name	IC_{50} (µg/ml)
1	*Averrhoa carambola*	belimbing besi	59.50
2	*Allium fistulosum*	Daun bawang	77.50
3	*Vigna sinensis*	kacang panjang	83.50
4	*Centella asiatica*	pegaga	90.00
5	*Allium tuberosum*	Ku chai	90.80
6	*Hibiscus esculentus*	bendi	93.00
7	*Citrus aurantifolia*	limau	96.00
8	*Luffa acutangula*	petola segi, ketola segi	96.80
9	*Apium graveolens*	selderi	97.50
10	*Amaranthus gangeticus*	bayam merah	>100
11	*Amaranthus viridis*	bayam pasir	>100
12	*Archidendron jiringga*	jering	>100
13	*Asparagus officinalis*	Asparagus, saparu keras	>100
14	*Beta vulgaris*	bit	>100
15	*Brassica oleracea* var. *alboglabra*	Kai lan	>100
16	*Capsicum annum*	lada merah,	>100
17	*Capsicum frutescens*	chabai, lada api, lada kerawit	>100
18	*Cosmos caudatus*	ulam rajah	>100
19	*Cucumis sativus*	timun, timun China, mentimun	>100
20	*Cucurbita moschata*	labu merah, labu perang,	>100
21	*Daucus carota*	lobak merah	>100

Table 4.9 continued

22	*Ipomoea batatas*	keledek	>100
23	*Ipomoea reptans*	kangkung	>100
24	*Lactuca sativa*	salad, selada	>100
25	*Lagenaria sceraria*	labu ayer putih, labu botol	>100
26	*Lycopersicum esculentum*	tomato	>100
27	*Manihot esculenta*	ubi kayu	>100
28	*Momordica charantia*	peria	>100
29	*Neptunia prostrata*	tangki,	>100
30	*Oenanthe javanica*	shelum	>100
31	*Pachyrrhizus erosus*	sengkuwang,	>100
32	*Parkia speciosa*	petai	>100
33	*Petroselium crispum*	parsley	>100
34	*Phaseolus vulgaris*	kacang buncis	>100
35	*Pisum sativum*	kacang puteh	>100
36	*Portulaca oleracea*	beremi	>100
37	*Psophocarpus tetragonolobus*	kacang botor,	>100
38	*Sesbania grandiflora*	turi	>100
39	*Solanum melongena*	terong	>100
40	*Solanum tuberosum*	ubi benggala, ubi kentang, ubi gendang	>100

4.3 Anti-HPV 16 E6 Oncoprotein Activity in Selected Vegetable

The crude methanol and water extracts from 10 selected vegetables were analyzed qualitatively for their anti-HPV 16 E6 protein activity in HPV 16-containing cervical cancer-derived cell line, CaSki. The CaSki cells were treated with the vegetable extracts at various concentrations (1, 10, 25, 50 and 100 μg/ml) for 3 days. The immunocytochemistry technique, 3-step Indirect Avidin-Biotin Immunoperoxidase with the anti-HPV 16 monoclonal antibody were successfully applied in this study to analyse the expression of E6 HPV 16 oncoprotein in treated and untreated CaSki cells.

Presence or reddish-brownish coloured products in the cytoplasmic and/or nuclear regions indicate expression/ presence of the HPV 16 E6 oncoprotein. The higher the intensity of the coloured products, the higher the amounts of the protein. On the other hand, the lower the amount of E6 simultaneously suggested evidence of suppression by the vegetable extracts. The staining intensity was classified as no stain (-), very weak (+), weak (2+), moderate (3+), strong (4+) and very strong (5+) as illustrated in Figure 4.9.

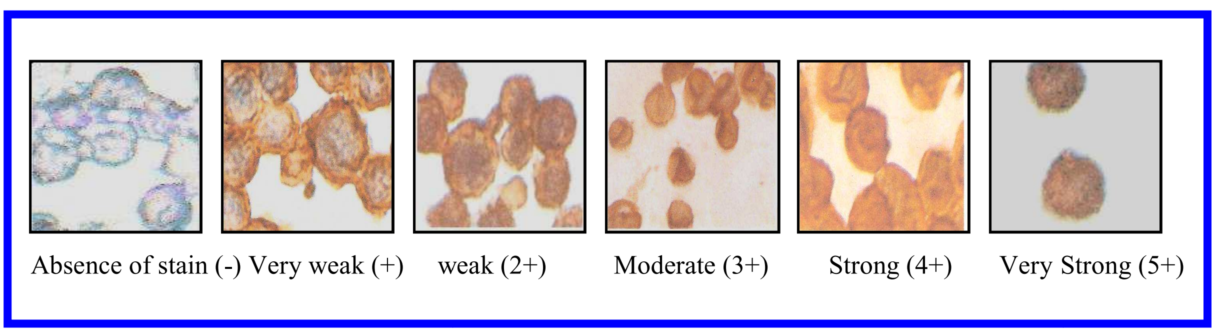
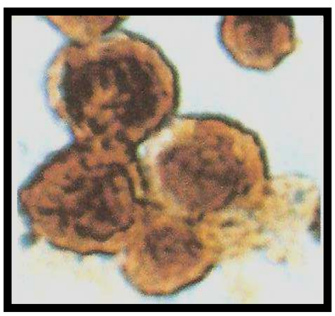
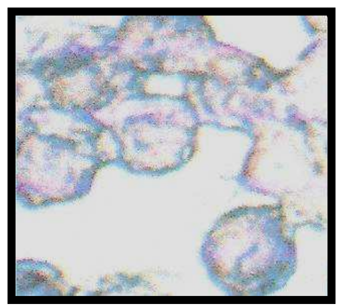


Figure 4.9: Staining intensities of HPV 16 E6 oncoprotein in CaSki cells using the 3-step Indiret Avidin-Biotin Immunoperoxidase methods.

In this present study, two types of negative controls were used. The CaSki cells not treated with the extracts and incubated with anti-HPV 16 E6 monoclonal antibody and CaSki cells not treated with the extracts and not incubated with anti-HPV 16 E6 monoclonal antibody. The staining results for untreated CaSki cells with and without anti-HPV monoclonal antibody are shown in Figures 4.10(a) and 4.10(b). All untreated CaSki cells appeared to be morphologically intact. The negative control incubated with anti-HPV monoclonal antibody exerted very strong reddish-brown stain (5+) due to the detection of high expression of E6 protein while no stain (-) was observed for untreated CaSki cells without incubation with anti-HPV 16 E6 monoclonal antibody



(a)



(b)

Figure 4.10 (a): Untreted CaSki cells incubated with anti-HPV 16 E6 monoclonal antibody. Very strong staining (5+) was observed in the nuclear and cytoplasmic regions (400X)

Figure 4.10 (b): Untreated without incubation with anti-HPV 16 E6 monoclonal antibody. No staining (-) was observed (400X)

Expressions of HPV 16 E6 protein after treatment with varying concentrations of vegetable extracts are shown in Figures 4.11-4.20. The staining intensities of the CaSki cell treated with different extracts at various concentrations were compared with each other and with the negative control. The morphology of the cells and the intensity of the coloured products were analysed.

4.3.1 *Asparagus officinalis*

As seen in Figure 4.11, the intensity of reddish-brown stain in CaSki cells treated with methanol and water extracts of *Asparagus officinalis* decreased with increasing concentrations of the vegetable extracts. This suggest suppression of HPV 16 E6 oncoprotein in CaSki cells treated with *A. officinalis* extracts at concentration as low as 1 µg/ml. Suppression increased with increasing concentration of the methanol and water extracts of *A. officinalis*.

The methanol extracts of *A. officinalis* produced just a weak suppression of HPV 16 E6 oncoprotein at 1 µg/ml and 10 µg/ml. The suppression increased then on to moderate at 25 µg/ml, strong at 50 µg/ml and became very strong at 100 µg/ml. In contrast, the weak suppression of HPV 16 E6 oncoprotein was observed in CaSki cells treated with water extracts of *A.officinalis* at 1 µg/ml, 10 µg/ml, 25 µg/ml and 50 µg/ml butbecame very strong at 100 µg/ml.

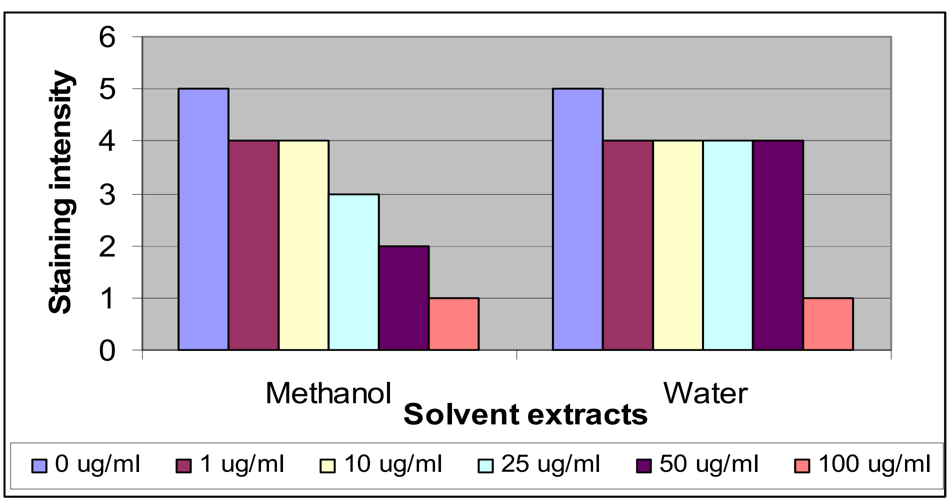


Figure 4.11: Staining intensities of Caski cells treated with *Asparagus officinalis*
Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.2 *Beta vulgaris*

Figure 4.12, illustrates a reduction in intensity of the reddish-brown stain in CaSki cells treated with methanol and water extracts of *Beta vulgaris.* The expression of HPV 16 E6 oncprotein decreased with increasing concentrations of the *B. vulgaris*. The methanol extracts of *B. vulgaris* exhibited moderate suppression of HPV 16 E6 oncprotein at 1 µg/ml and 10 µg/ml. However from then suppression of HPV 16 E6 in Caski cells treated with methanol extracts of *B. vulgaris* became very strong at 25 µg/ml, 50 µg/ml and 100 µg/ml.

Overall indicated that the water extracts of *Beta vulgaris* was slightly more suppressive against HPV 16 E6 oncoprotein at 10 µg/ml as compared to methanol counterparts. In general, water extracts of *B. vulgaris* exhibited moderate suppression at 1 µg/ml, and stronger at 10 µg/ml and became very strong at 25 µg/ml, 50 µg/ml and 100 µg/ml. CaSki cells treated with *B. vulgaris* extracts remain morphologically intact at all concentrations.

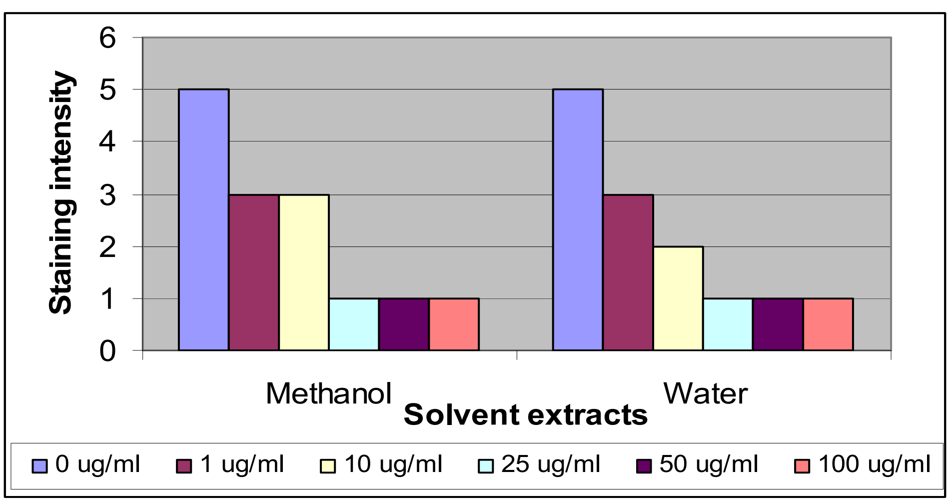


Figure 4.12: Staining intensities of Caski cells treated with *Beta vulgaris*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.3 *Ipomea batatas*

Figure 4.13 shows that, a reduction in intensity of the reddish-brown stain in CaSki cells treated with methanol and water extracts of *I. batatas.* Expression of HPV 16 E6 oncoprotein decreased with increasing concentrations of *I. batatas* extracts. The methanol extracts of *I. batatas* exhibited very strong suppression of HPV 16 E6 oncoprotein at all concentrations tested.

In contrast, the water extracts of *I. batatas* inhibited weak suppression of HPV 16 E6 oncoprotein at 1 µg/ml and 10 µg/ml but became strong at 25 µg/ml, 50 µg/ml and 100 µg/ml. In general, CaSki cells treated with *I. batatas* extracts remained morphologically intact at all concentrations.

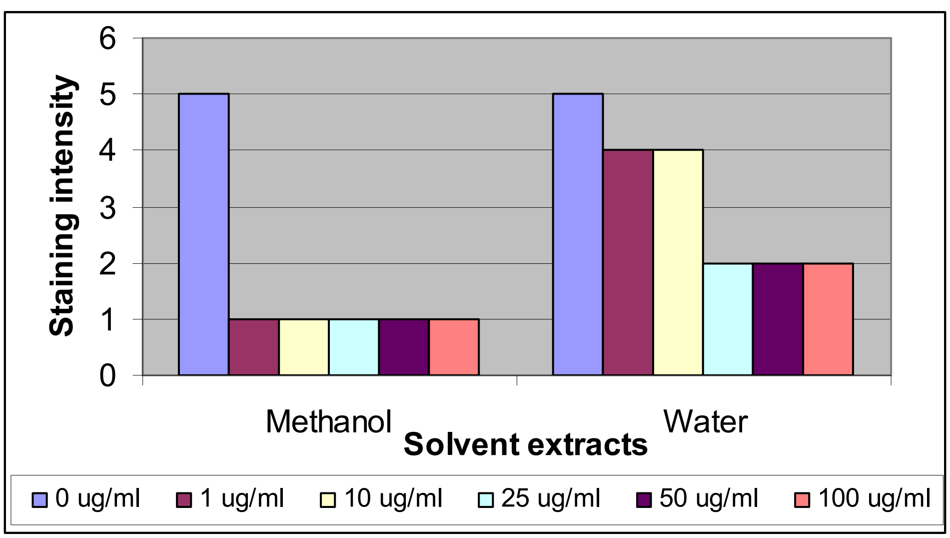


Figure 4.13: Staining intensities of Caski cells treated with *Ipomea batatas*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.4. *Manihot esculentus*

Figure 4.14, shows that methanol and water extracts of the *M. esculentus* exerted inhibition activity against the HPV-16 E6 oncoprotein. All CaSki cells treated with *M. esculentus* extracts remained morphologically intact at all concentrations used.

In general**,** the *M. esculenta* extracts demonstrated good inhibition activities against HPV16 E6 oncoprotein in CaSki cells. The methanol extracts of *M. esculenta* exhibited strong suppression of HPV 16 E6 oncoprotein at concentrations 1 µg/ml and 10 µg/ml. Suppression of HPV 16 E6 became very strong at concentrations 25 µg/ml, 50µg/ml and 100 µg/ml. In contrast, the water extract of *M. esculentus* exhibited strong suppression of HPV 16 E6 oncoprotein at all concentrations tested.

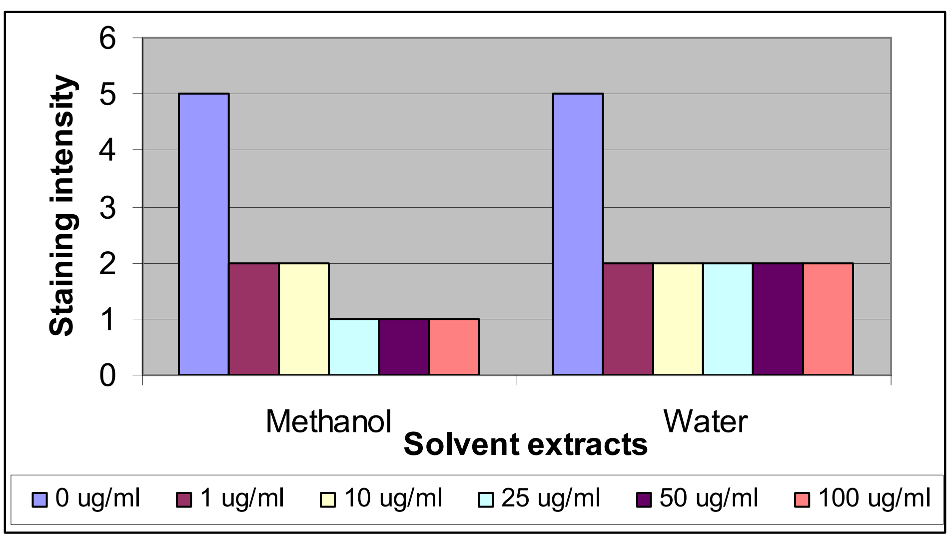


Figure 4.14: Staining intensities of Caski cells treated with *Manihot esculentus*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.5 *Pachyrrizus erosus*

Figure 4.15 show that, the intensities of reddish-brown stain decreased with increasing concentrations of methanol and water extracts of *P. erosus*. This shows that the expression of HPV-16 E6 oncoprotein in CaSki cells reduced when the concentration of *P. erosus* extracts increased. Overall results showed that the reddish brown stain was seen in both nuclear and cytoplasmic regions of CaSki cells. The methanol extract of *P. erosus* produced no suppression of HPV 16 E6 at 1 µg/ml, but produced weak suppression at 10 µg/ml. The suppression increased then on to moderate at 25 µg/ml and became strong at 50 µg/ml and 100 µg/ml.

In contrast, the water extract of *P. erosus* exhibited moderate suppression inhibition of HPV 16 E6 oncoprotein at concentrations as low as 1 µg/ml. Suppression of HPV 16 E6 became strong at 10 µg/ml and 25 µg/ml. The inhibition became very strong then on at concentrations 50 µg/ml and 100 µg/ml. The CaSki cells treated with methanol extract started to lysie at concentrations 25 µg/ml to 100 µg/ml. In contrast, the CaSki cells remained morphological intact when treated with water extracts at all of concentrations used.

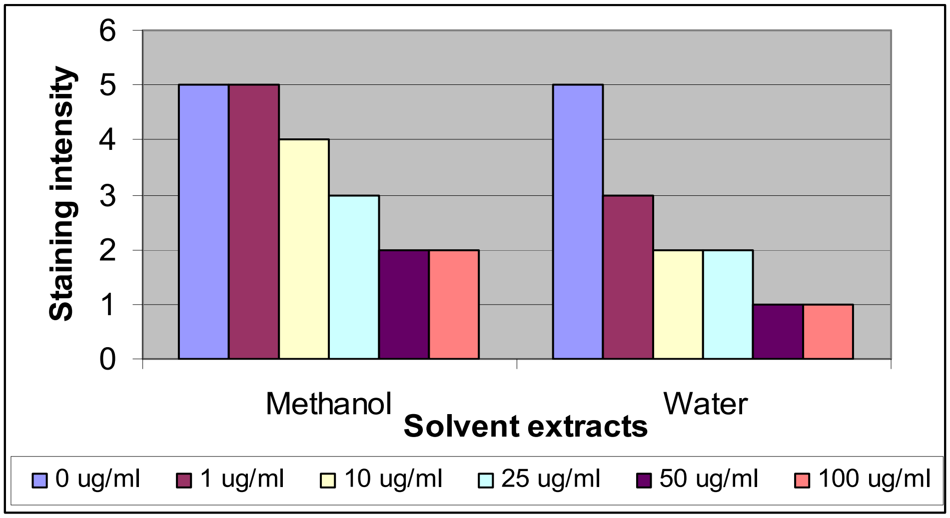


Figure 4.15: Staining intensities of Caski cells treated with *Pachyrrizus erosus*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.6 *Pertroselium crispum*

As seen in Figure 4.16, there is reduction in intensity of the reddish-brown stain at concentrations as low as 1 µg/ml for methanol and water extracts of *P. crispum*. Very weak suppression of HPV 16 E6 oncoprotein was observed in CaSki cells treated with methanol extracts at all concentrations tested and in CaSki cells treated with water extracts at concentrations 1 µg/ml, 25 µg/ml and 50 µg/ml. The suppression of HPV 16 E6 protein became moderate in cells treated with 100 µg/ml of the water extract. The integrity of CaSki cells treated with *P. crispum* extracts were negatively affected where the cells lysed at concentrations as low as 1 µg/ml (for methanol and water extract) and 10 µg/ml. The distribution of stain was observed either in the cell cytoplasm only or both nucleus and cytoplasm.

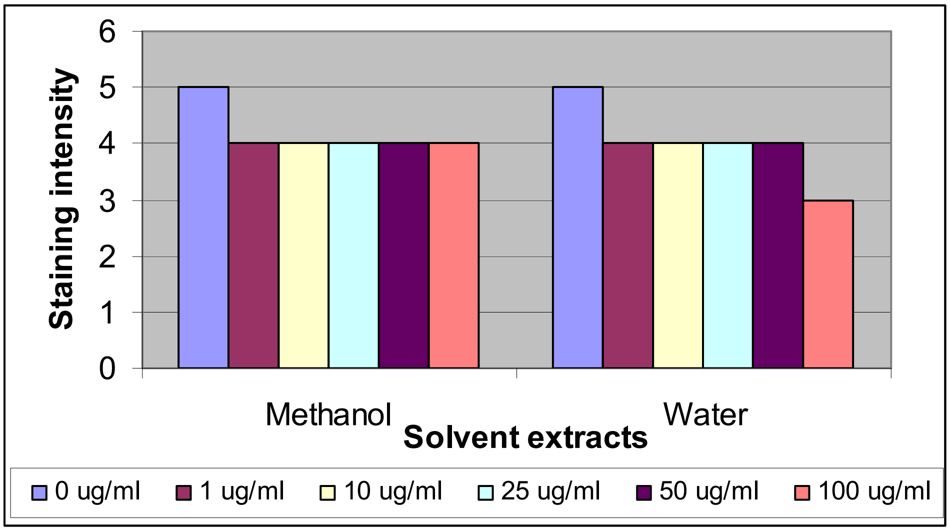


Figure 4.16: Staining intensities of Caski cells treated with *Petroselium crispum*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.7 *Phaseolus vulgaris*

Figure 4.17, illustrates that the methanol extract of *P. vulgaris* exhibited very strong suppression of HPV 16 E6 at 1 µg/ml and 10 µg/ml. However, the suppression weakered from then on resulting in the reddish-brown stain to become stronger with increasing concentrations of the *P. vulgaris* methanol extract.

In contrast, the water extracts did not exhibit any suppression of HPV 16 E6 oncoprotein at 1 µg/ml and 10 µg/ml. Weak suppression was seen at 25 µg/ml, suppression of HPV 16 E6 become strong at 50µg/ml and very strong at 100 µg/ml. Cell lysis was observed at 1 µg/ml for water extracts and at concentration as low as 25 µg/ml for methanol extracts.

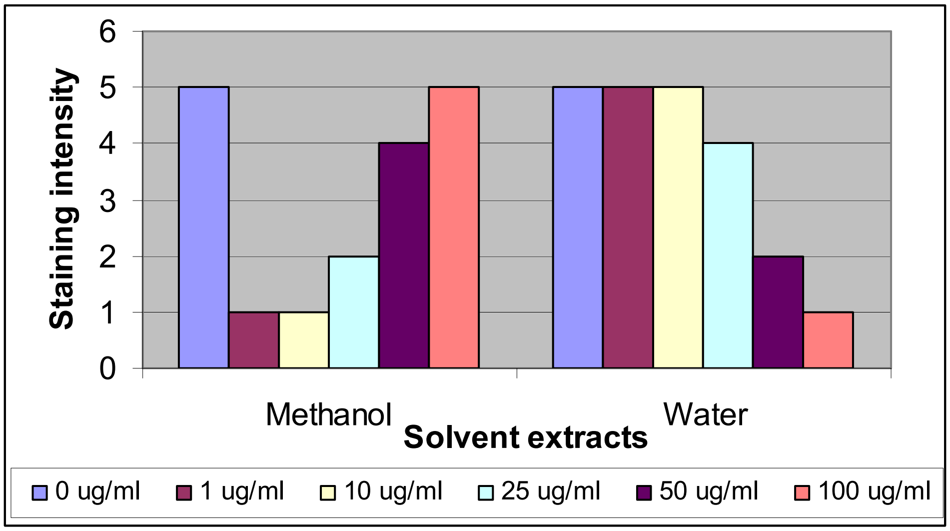


Figure 4.17: Staining intensities of Caski cells treated with *Phaseolus vulgaris*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.8 *Portulaca oleracea*

The resulting staining intensity of CaSki cells treated with *Portulaca oleracea* extracts are shown in Figure 4.18**.** In general**,** the *P. oleracea* extracts demonstrated good inhibition activities against HPV16 E6 oncoprotein in CaSki cell. The methanol extract of *P. oleracea* very strongly suppressed HPV 16 E6 oncoprotein at concentrations 1 µg/ml, 10 µg/ml and 25 µg/ml. However, the inhibition weakered a little (though still strong) at concentrations 50 µg/ml and 100 µg/ml.

In contrast, the water extract of *P. oleracea* produced strong inhibition of HPV 16 E6 oncoprotein at concentrations as low as 1 µg/ml and 10 µg/ml. The inhibition then became very strong at higher concentrations. All the CaSki cells treated with *P. oleracea* extracts appeared to be morphologically intact. Overall results showed that the reddish brown stain was seen in both nuclear and cytoplasmic regions.

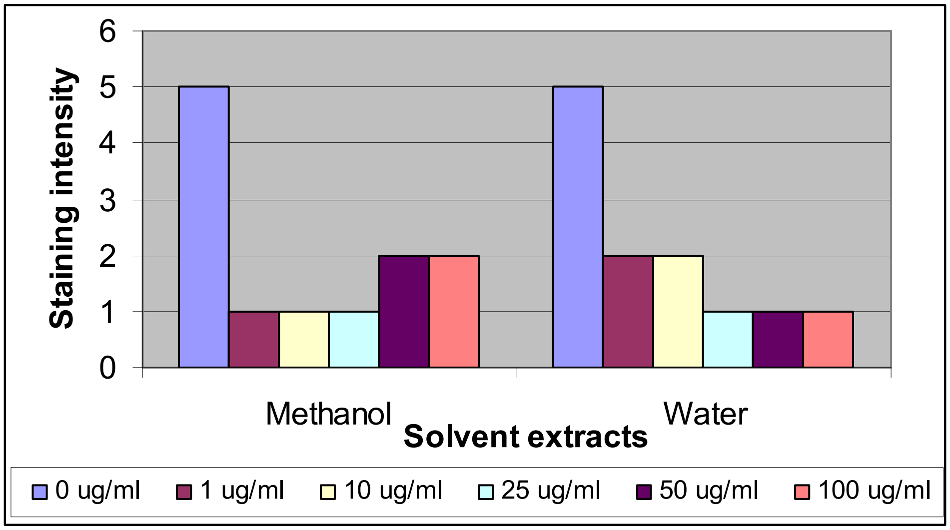


Figure 4.18: Staining intensities of Caski cells treated with *Portulaca oleracea*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.9 *Sesbania grandiflora*

As denoted by the reduction of reddish-brown stain in CaSki cells, the inhibition activities of HPV 16-E6 oncoprotein by *S. grandiflora* extracts increased with the increasing concentrations of the extracts (Figure 4.19). The methanol extract of *S. grandiflora* produced a strong inhibition of HPV 16 E6 oncoprotein at concentrations as low as 1 µg/ml and remained consistent at concentrations 10 µg/ml and 25 µg/ml. The inhibition became very strong from then on at concentrations 50 µg/ml and 100 µg/ml.

In contrast the water extract of *S. grandiflora* produced a weak suppression of HPV 16 E6 at 1 µg/ml. The suppression increased then on to moderate at 10 µg/ml, strong at 25 µg/ml and 50 μg/ml and became very strong at 100 μg/ml. Evident here that methanol extract produced a greater inhibition effects as compared to water extract. The integrity of CaSki cells were negatively affected where the cells lysed after treated with methanol at 25 μg/ml and 50 μg/ml.

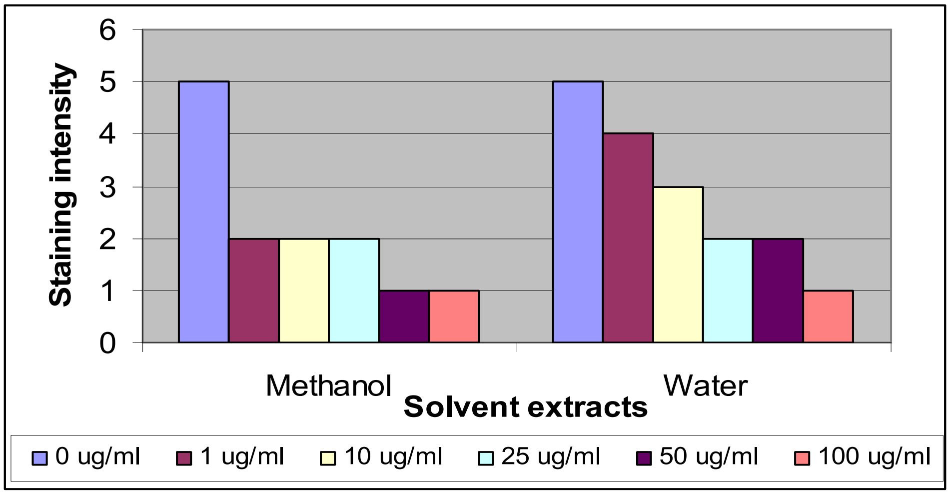


Figure 4.19: Staining intensities of Caski cells treated with *Sesbania grandiflora*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.3.10 *Solanum tuberosum*

The resulting staining intensity of the CaSki cells treated with *Solanum tuberosum* extracts are shown in Figure 4.20. The decrease in staining intensity was observed in CaSki cells treated with increasing concentration of the extracts. This indicates that the *S. tuberosum* extracts inhibited the expression of HPV 16 E6 oncoprotein in a dose-dependent manner, where the suppressing effects increased with increasing concentration of the extracts.

The water extracts of *S. tuberosum* exerted a more suppressive effect against HPV-16 E6 oncoprotein as compared to methanol extracts. Both extracts exerted weak suppressing effects against HPV 16 E6 oncoprotein at concentrations 1 µg/ml and 10 µg/ml. However, from then on CaSki cells treated with water extracts exhibited very weak reddish brown stain, indicating very strong suppression at concentrations, 25 µg/ml, 50 µg/ml and 100 µg/ml. Methanol extracts of *S. tuberosum* on the other hand produced just moderate suppression of the oncoprotein at concentrations, 25 µg/ml, 50 µg/ml and 100 µg/ml. The integrity of CaSki cells treated with *S. tuberosum* extracts were negatively affected where the cells lysed at concentration as low as 25 µg/ml (for methanol extract).

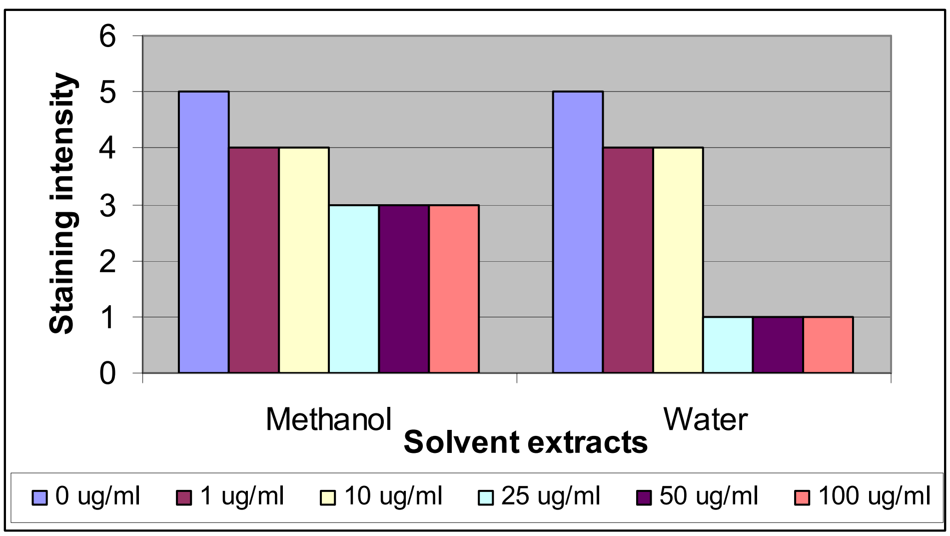


Figure 4.20: Staining intensities of Caski cells treated with *Solanum tuberosum*

Classification for staining intensity as: no stain (0), very weak (1+), weak (2+), moderate (3+), strong (4+) and very strong (5+)

4.4 Comparison of Anti-HPV 16 E6 Oncoprotein Activity in Selected Vegetable

Twenty crude extracts (methanol and water) from ten selected vegetables were analyzed qualitatively for possible anti-HPV 16 E6 oncoprotein activity. The staining intensity of coloured products was observed in CaSki cells treated with the selected vegetable crude extracts indicated that each vegetable extracts possess different ability in suppressing the expression of HPV 16 E6 oncoprotein (Figure 4.21 and Figure 4.22).

Overall results indicate that not all of the vegetable extracts suppressed the HPV 16 E6 oncoprotein in a dose-dependent manner where greater inhibition activity against E6 oncoprotein was observed in higher extract concentration.

Ten out of 20 extracts (50%) were found effective in suppressing the HPV 16 E6 oncoprotein at the lowest extract concentration (25 µg/ml and below) used in this study. They were both methanol and water extracts for *P. oleracea*, *I. batatas*, *M. esculentus*, methanol extract for *P. erosus*, *B. vulgaris*, *S. grandiflora* and water extracts for *A. officinalis*.

Overall results indicated that *P. oleracea* extracts was the most effective in suppressing the HPV 16 E6 oncoprotein. When compared, water extracts was better than methanol extracts at suppressing the HPV 16 E6 oncoprotein.

Appearance of CaSki cells after treatment with methanol extract of *Asparagus officinalis*

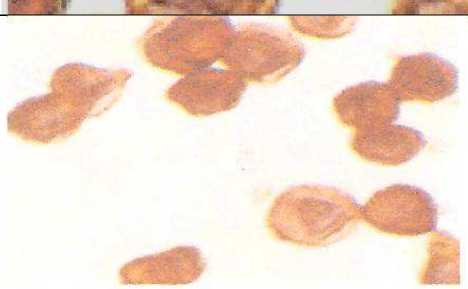
	Concentration of extracts (µg/ml)	Morphology of cells	Intensity of reddish-brown stain
	100	Intact	+
	50	Majority intact but some showing lysis	2+
	25	Majority intact but some showing lysis	2+
	10	Intact	4+
	1	Intact	4+

Figure 4.21: CaSki cells treated with methanol extracts of *Asparagus officinalis* (400x)
Note: Classification for the intensity of staining as no stain (-), very weak (+), weak (2+), moderate (3+), strong (4+) and very strong (5+

Appearance of CaSki cells after treatment with water extract of *Solanum tuberosum*

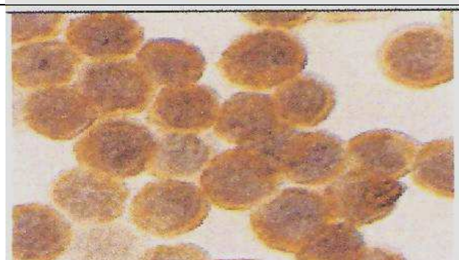
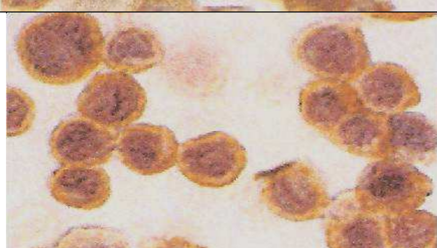
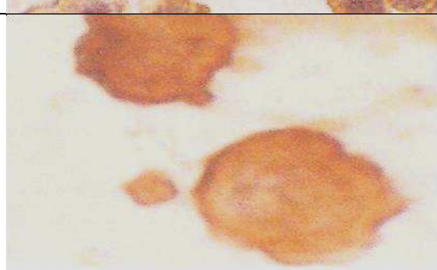
	Concentration of extracts (µg/ml)	Morphology of cells	Intensity of reddish-brown stain
	100	Intact	+
	50	Intact	+
	25	Intact	+
	10	Intact	4+
	1	Intact	4+

Figure 4.22: CaSki cells treated with water extracts of *Solanum tuberosum* (400x)

Note: Classification for the intensity of staining as no stain (-), very weak (+), weak (2+), moderate (3+), strong (4+) and very strong (5+)