4.0 RESULTS

The petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum*, *Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus* and *Sauropus androgynus* were evaluated for their antioxidant potentials using DPPH Radical Scavenging Assay, Reducing Power Assay, Metal Chelating Assay, Haemolysate Catalytic Assay and Lipid Hydroperoxide Assay.

4.1 DPPH radical scavenging assay

4.1.1 Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical

The free radical scavenging activity of crude petroleum benzene, chloroform, methanol and water extract of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus* and *Sauropus androgynus* were measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Positive DPPH test suggests that the samples contain free radical scavengers or antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The plant extracts were evaluated at varying concentrations of 0.0025 mg/ml, 0.0125 mg/ml, 0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.

4.1.2 Ascorbic acid as positive references standard

Ascorbic acid is a potent antioxidant known to scavenge a wide variety of reactive oxygen species (ROS). In this DPPH radical scavenging assay, ascorbic acid was used as reference standard compounds. Table 4.1 shows average percentages of inhibition of DPPH radical by ascorbic acid at different concentrations ranging from 0.0025 mg/ml, 0.0125 mg/ml, 0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml to 0.5 mg/ml.

The percentage of inhibition of DPPH radical by the ascorbic acid increased with increasing concentrations of ascorbic acid. The percentage of DPPH radical scavenging activity at 0.0025 mg/ml, 0.0125 mg/ml, 0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml were 9.66 ± 2.64%, 91.78 ± 1.78%, 96.58 ± 0.85%, 96.89 ± 0.79%, 97.39 ± 0.51% and 98.03 ± 0.38% respectively.

The percentage of inhibition values were used to plot a dose-response curve (Figure 4.1). IC_{50} is the concentration at which 50% of DPPH radicals are inhibited. The percentage of inhibition increased sharply until 0.0125 mg/ml of ascorbic acid. After that, the increment reduced until 0.5 mg/ml. This means that the scavenging effect increased with increasing concentration of the ascorbic acid. IC_{50} value was obtained at 0.00375 mg/ml. Ascorbic acid was able to reduce and bleach the colour of DPPH solution from purple to the yellow colour diphenylpicrylhydrazine. Mean values of the reading or optical density at 517 nm from three independent test runs were used to calculate these average percentage of inhibition.

Table 4.1: Percentage of inhibition (%) of DPPH radical by ascorbic acid

Concentration of ascorbic acid(mg/ml)	Average Percentage of inhibition(POI)
CONTROL	0.00±0.00
0.0025	9.66±2.64
0.0125	91.78±1.78
0.0375	96.58±0.85
0.125	96.89±0.79
0.25	97.39±0.51
0.5	98.03±0.38

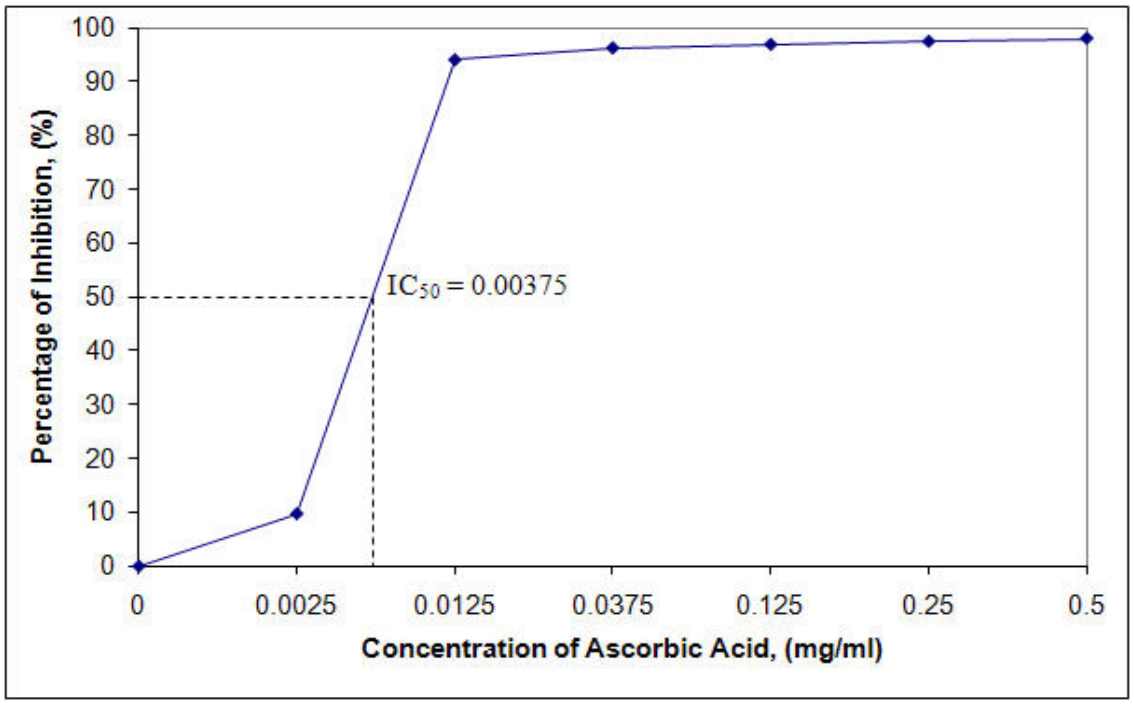


Figure 4.1: The dose-response curve of ascorbic acid as positive reference standard in the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

4.1.3 Screening of DPPH radical scavenging potential of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus* and *Sauropus androgynus* extracts

Crude petroleum benzene, chloroform, methanol and water extracts of of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* were evaluated for their potential to scavenge DPPH radicals at 0.0025 mg/ml, 0.0125 mg/ml, 0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml. The average percentage of inhibition from

screening of DPPH radical scavenging activity by the 24 crude extracts tested are shown in Tables 4.2 - 4.7. Mean values of the absorbance reading or optical density at 517 nm from three independent test runs were used to calculate these average percentage of inhibition.

Table 4.2: Average percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum*

Concentration of crude extract (mg/ml)	Percentage of inhibition (%) for Garlic Chives			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	5.40±2.55	4.40±0.65	5.84±3.13	9.42±0.36
0.0125	13.36±1.62	29.06±4.72	10.77±3.86	17.25±1.29
0.0375	18.80±2.88	36.05±1.60	16.40±1.80	28.38±1.42
0.125	21.63±2.55	42.42±2.12	20.94±3.34	43.02±0.99
0.25	28.82±2.11	43.57±1.84	28.16±0.91	55.39±0.49
0.5	36.62±1.22	51.28±3.96	43.54±2.07	68.10±0.24

Table 4.3: Percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.)

Concentration of crude extract (mg/ml)	Percentage of inhibition (%) for Celery			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	11.56±0.92	3.75±0.73	15.20±0.16	10.75±0.55
0.0125	20.63±0.62	11.56±0.88	17.95±1.06	41.88±0.67
0.0375	27.54±0.30	22.50±0.64	26.75±0.09	57.22±0.33
0.125	32.12±0.92	33.29±0.39	30.33±0.35	68.09±0.34
0.25	35.39±1.06	42.61±0.43	33.87±0.78	84.10±0.60
0.5	49.81±0.26	46.53±0.62	42.81±0.59	87.91±0.24

Table 4.4: Percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Ipomoea batatas* (L.)

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Sweet Potato Leaves			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	2.85±1.89	4.89±0.53	11.17±4.49	7.72±1.03
0.0.125	3.69±1.87	6.11±0.62	14.50±4.01	11.12±1.14
0.0375	10.04±1.84	8.89±4.03	21.78±5.70	42.27±0.74
0.125	24.24±2.30	10.44±4.37	25.28±2.38	60.50±0.84
0.25	29.12±0.51	24.33±7.17	42.52±4.50	69.35±0.30
0.5	39.21±2.17	50.22±1.26	83.16±3.69	79.00±0.47

Table 4.5: Percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.)

Concentration of crude extract (mg/ml)	Percentage of inhibition (%) for Curry Leaves			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	33.69±1.80	15.19±2.95	8.86±4.72	13.30±1.37
0.0125	44.19±1.70	26.22±1.43	24.49±1.17	24.36±0.70
0.0375	58.07±6.73	65.93±2.21	28.38±2.36	56.65±0.52
0.125	86.11±0.77	75.62±1.97	37.30±2.82	65.81±0.49
0.25	86.70±0.75	82.48±1.00	42.67±4.01	85.47±0.49
0.5	89.20±0.30	88.65±0.43	44.1±3.37	87.04±0.27

Table 4.6: Percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus*

Concentration of crude extract (mg/ml)	Percentage of inhibition (%) for Winged Bean			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	2.20±0.76	8.13±0.69	11.97±2.81	21.17±0.91
0.0125	30.85±0.29	11.29±0.89	17.80±3.75	28.60±0.89
0.0375	36.28±0.18	23.77±0.95	22.69±4.53	57.89±0.58
0.125	42.35±0.36	29.10±0.96	52.41±2.64	72.31±0.24
0.25	45.40±0.41	33.04±0.62	71.58±0.52	87.19±0.79
0.5	52.42±0.76	35.83±0.82	84.77±0.98	92.11±0.94

Table 4.7: Percentage of inhibition (%) of DPPH radical by crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus*

Concentration of crude extract (mg/ml)	Percentage of inhibition (%) for Sweet Leaves			
	Petroleum Benzene	Chloroform	Methanol	Water
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0025	8.10±0.81	9.09±0.59	2.67±0.54	13.45±0.77
0.0125	29.45±0.78	32.11±0.38	33.11±0.64	22.94±0.69
0.0375	50.59±1.02	42.33±0.28	41.53±1.00	34.80±0.81
0.125	52.45±0.95	50.50±0.72	48.28±0.58	43.39±0.48
0.25	71.29±0.70	51.72±0.55	58.58±0.70	47.57±0.84
0.5	86.30±1.02	67.49±0.46	67.66±0.57	56.27±0.79

Crude extracts showing percentage of inhibition of more than 50% was considered as positive extracts for the radical scavenging activity. The percentage of inhibition values obtained from the petroleum benzene, chloroform, methanol and water extracts of each selected local vegetables were plotted out into dose response curves and compared with ascorbic acid standard as shown in Figures 4.2-4.7. IC_{50} is the concentration at which 50% of DPPH radicals are inhibited. The IC_{50} for each positive crude extract was extrapolated and determined from the respective dose-response curve. Out of the 24 extracts tested, 16 crude extracts showed satisfactory DPPH radical scavenging capabilities by exhibiting more than 50% of inhibition at varying concentrations ranging from 0.0375 mg/ml to 0.5 mg/ml while the remaining 8 crude extracts, that is petroleum benzene, chloroform and methanol extract of *Apium graveolens* (L.); petroleum benzene and methanol extract of *Allium tuberosum;* petroleum benzene extract of *Ipomoea batatas* (L.); methanol extract of *Murraya koenigii* (L.) and chloroform extract of *Psophocarpus tetragonolobus* showed percentage of inhibition of less than 50% even at the highest concentration tested that is 0.5mg/ml. Thus, the IC_{50} of these 8 crude extracts could not be determined from the concentrations evaluated in this study. In comparison to the positive reference standard ascorbic acid, the radical scavenging activities of these extracts are very low.

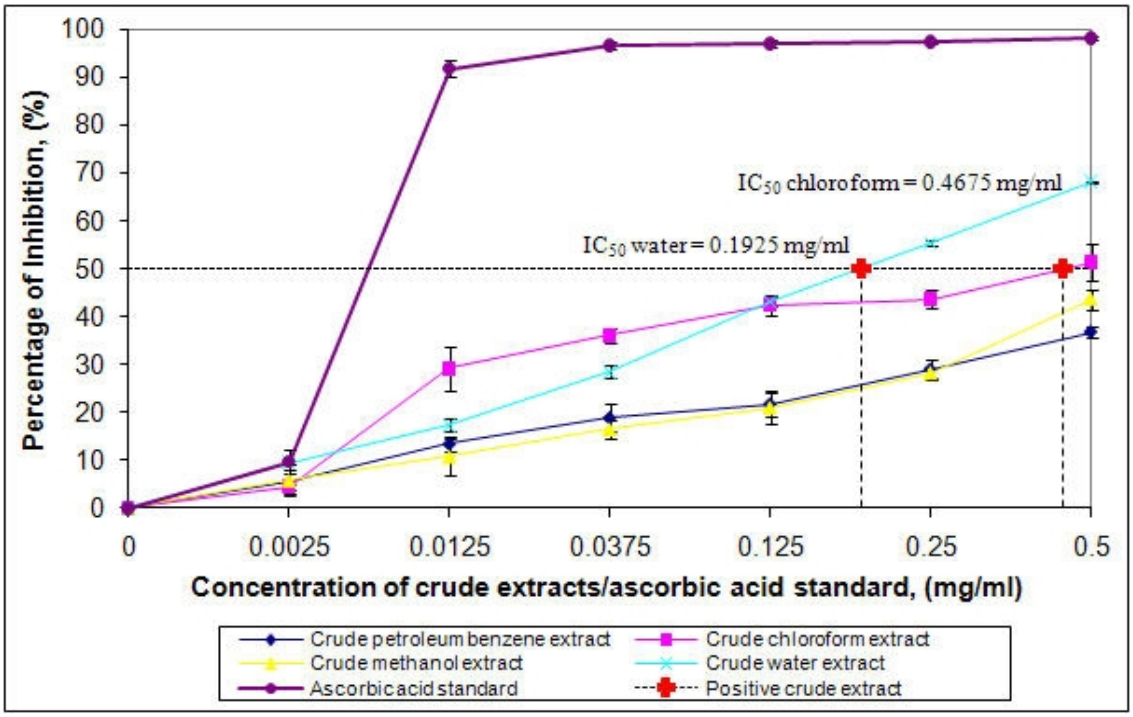


Figure 4.2: Dose-response curves showing scavenging activities of *Allium tuberosum* extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

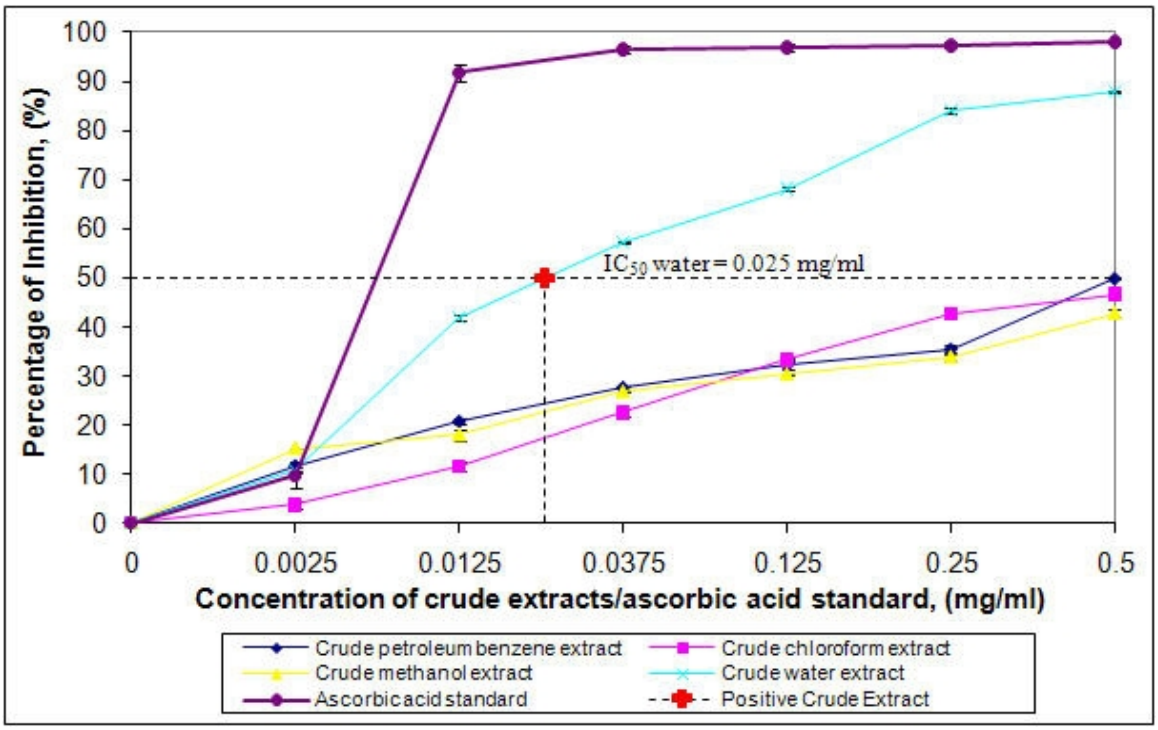


Figure 4.3: Dose-response curves showing scavenging activities of *Apium graveolens* (L.) extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

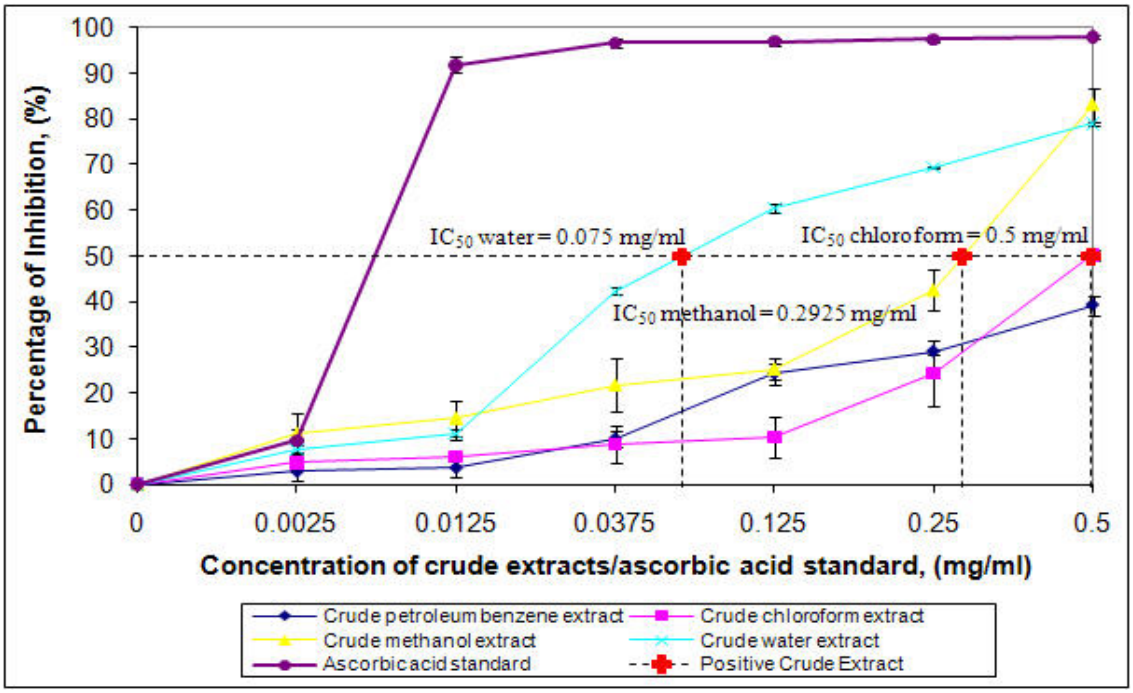


Figure 4.4: Dose-response curves showing scavenging activities of *Ipomoea batatas* (L.) extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

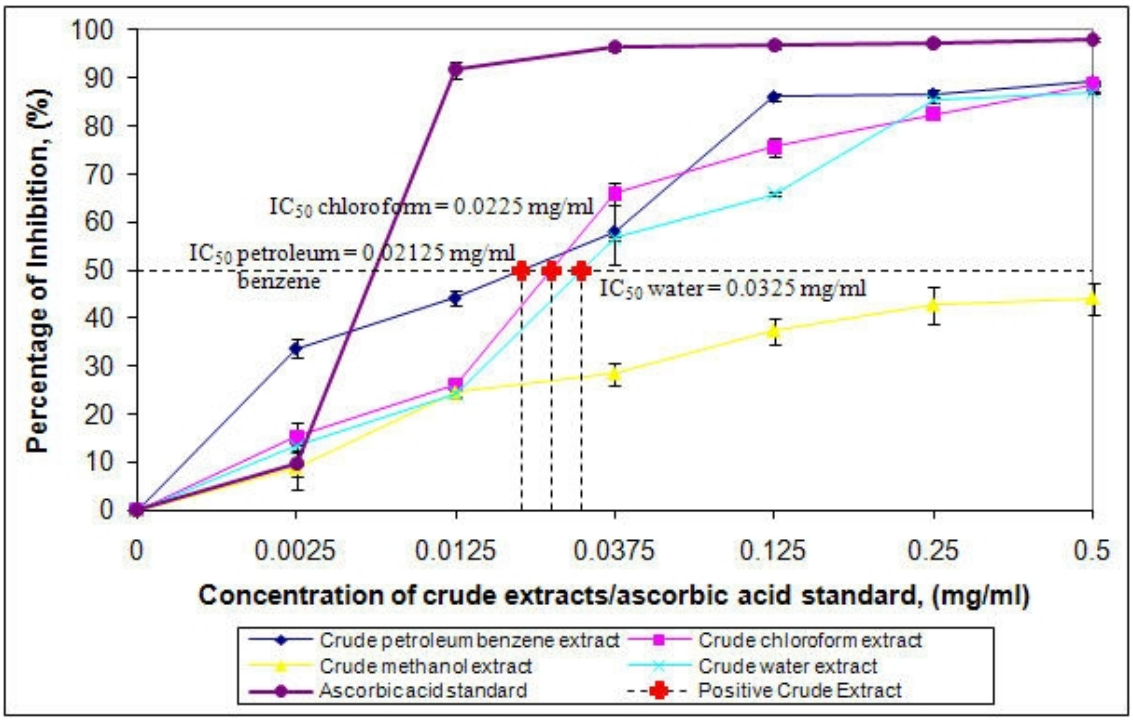


Figure 4.5: Dose-response curves showing scavenging activities of *Murraya koenigii* (L.) extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

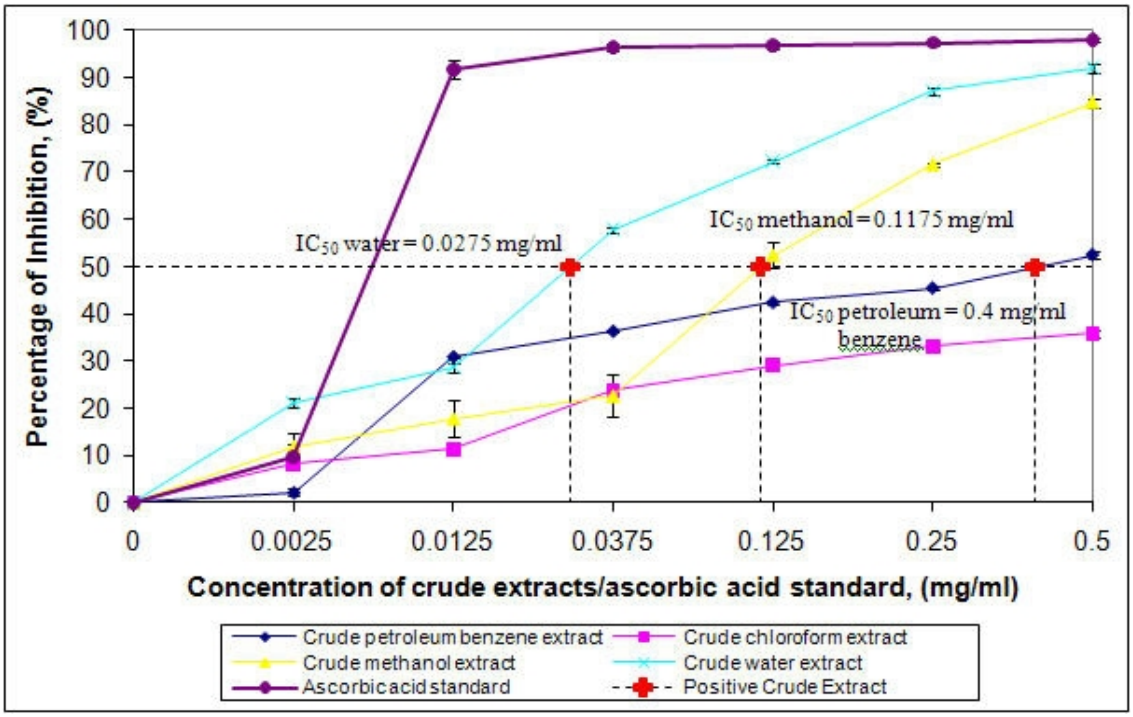


Figure 4.6: Dose-response curves showing scavenging activities of *Psophocarpus tetragonolobus* extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

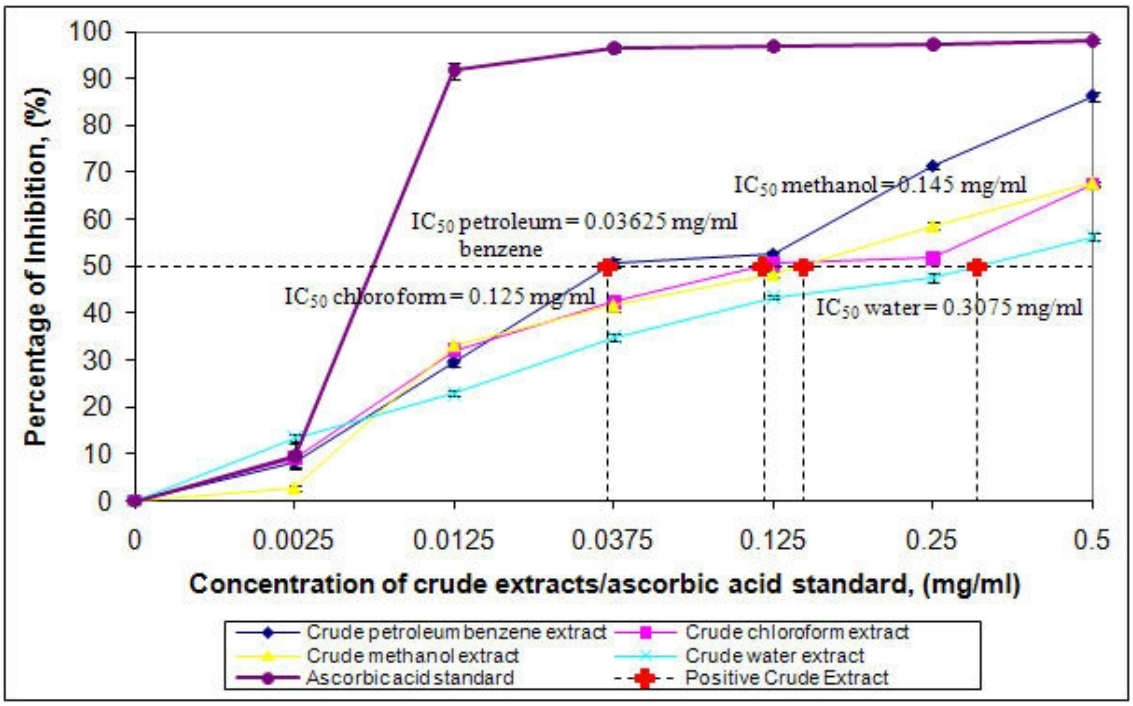


Figure 4.7: Dose-response curves showing scavenging activities of *Sauropus androgynus* extracts and compared with ascorbic acid as positive reference. Values are expressed as mean ± standard deviation of three independent experiments

(i) *Allium tuberosum*

- Chloroform extracts

As shown in Table 4.2 and illustrated in Figure 4.2, out of the 6 concentrations evaluated the crude chloroform extracts of *A. tuberosum* showed percentage of inhibition of **more than 50%** only at **0.5 mg/ml**. At that concentration, the extract inhibited DPPH radicals by **51.28 ± 3.96%.** The IC_{50} value extrapolated from the dose response curve is 0.4675 mg/ml or 467.5 µg/ml. This means that at that concentration, chloroform extracts of garlic chives inhibited 50% of DPPH radicals.

- Water extracts

As shown in Table 4.2 and illustrated in Figure 4.2, out of the 6 concentrations evaluated the crude water extracts of *A. tuberosum* showed percentage of inhibition of **more than 50%** at concentrations **0.25 mg/ml and 0.5 mg/ml.** At that concentration, the extract inhibited DPPH radicals by **55.39 ± 0.49%** and **68.10 ± 0.24%**, **respectively.** The IC_{50} value obtained from the dose response curve is 0.0325 mg/ml or 32.5 µg/ml. This means that at that concentration, water extracts of garlic chives inhibited 50% of DPPH radicals.

(ii) *Apium graveolens* (L.)

- Water extracts

As shown in Table 4.3 and illustrated in Figure 4.3, only crude water extracts of *A. graveolens* (L.) showed percentages of inhibition of more than 50%. This was achieved at concentrations of 0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml with **57.22 ± 0.33%, 68.09 ± 0.34%, 84.10 ± 0.60%, and 87.91 ± 0.24% inhibition, respectively.** The IC_{50} value extrapolated from the dose response curve is 0.025 mg/ml or 25 µg/ml.

This means that at that concentration, water extracts of *A. graveolens* (L.) inhibited 50% of DPPH radicals.

(iii) *Ipomoea batatas* (L.)

- Chloroform extracts

As shown in Table 4.4 and illustrated in Figure 4.4, out of the 6 concentrations evaluated crude chloroform extracts of ***I. batatas*** showed percentages of inhibition of more than 50% only at 0.5 mg/ml. At that concentration, the extract inhibited DPPH radicals by **50.22 ± 1.26%.** The IC_{50} value extrapolated from the dose response curve is 0.5 mg/ml or 500 µg/ml. This means that at that concentration chloroform extracts of sweet potato leaves inhibited 50% of DPPH radicals.

- Water extracts

As shown in Table 4.4 and illustrated in Figure 4.4, out of the 6 concentrations evaluated crude water extracts of ***I. batatas*** showed percentages of inhibition of more than 50% at the concentrations **0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml**. At those concentrations, the extract inhibited DPPH radicals by **60.50 ± 0.84%, 69.35 ± 0.30%, and 79.00 ± 0.47%, respectively.** The IC_{50} value extrapolated from the dose response curve is 0.075 mg/ml or 75 µg/ml. This means that at that concentration water extracts of sweet potato leaves inhibited 50% of DPPH radicals.

- Methanol extracts

As shown in Table 4.4 and illustrated in Figure 4.4, out of the 6 concentration evaluated, crude methanol extracts of ***I.batatas*** showed percentage of inhibition of more than 50% only at **0.5 mg/ml.** At that concentration, the extract inhibited DPPH radical by **83.16 ± 3.69%**. The IC_{50} value extrapolated from the dose-response curve is 0.0075

mg/ml or 7.5 µg/ml. This means that at that concentration, methanol extracts of sweet potato leaves inhibited 50% of DPPH radicals.

(iv) *Murraya koenigii* (L.)

- Petroleum benzene extracts

As seen in Table 4.5 and illustrated in Figure 4.5, out of the 6 concentrations evaluated the crude petroleum benzene extracts of *M. koenigii* showed percentages of inhibition of **more than 50%** at concentrations **0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **58.07% ± 6.73, 86.11 ± 0.77%, 86.70 ± 0.75% and 89.20 ± 0.30%, respectively.** The IC_{50} value obtained from the dose response curve is 0.02125 mg/ml or 21.25 µg/ml. This means that at that concentration petroleum benzene extracts of curry leaves inhibited 50% of DPPH radicals.

- Chloroform extracts

As seen in Table 4.5 and illustrated in Figure 4.5, out of the 6 concentrations evaluated the crude chloroform extracts of *M. koenigii* showed percentages of inhibition of **more than 50%** at concentrations **0.0375 mg/ml, 0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **65.93 ± 2.21%, 75.62 ± 1.97%, 82.48 ± 1.00% and 88.65 ± 0.43%, respectively.** The IC_{50} value obtained from the dose response curve is 0.0225 mg/ml or 22.5 µg/ml. This means that at that concentration chloroform extracts of curry leaves inhibited 50% of DPPH radicals.

(v) *Psophocarpus tetragonolobus*

- Petroleum benzene extracts

As seen in Table 4.6 and illustrated in Figure 4.6, out of the 6 concentrations evaluated the crude chloroform extracts of *P. tetragonolobus* showed percentages of inhibition of **more than 50%** only at the concentration **0.5 mg/ml.** At that concentration, the extract inhibited DPPH radicals by **52.42 ± 0.76%.** The IC_{50} value obtained from the dose response curve is 0.400 mg/ml or 400 µg/ml. At that concentration, petroleum benzene extracts of winged beans inhibited 50% of DPPH radicals.

- Methanol extracts

As seen in Table 4.6 and illustrated in Figure 4.6, out of the 6 concentrations evaluated the crude methanol extracts of *P. tetragonolobus* showed percentages of inhibition of **more than 50%** at concentrations **0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations the extract inhibited DPPH radicals by **52.41 ± 2.64%, 71.58 ± 0.52%, and 84.77 ± 0.98%, respectively**. The IC_{50} value obtained from the dose response curve is 0.1175 mg/ml or 117.5 µg/ml. At that concentration, methanol extracts of winged beans inhibited 50% of DPPH radicals.

- Water extracts

As seen in Table 4.6 and illustrated in Figure 4.6, out of the 6 concentrations evaluated the crude water extracts of *P. tetragonolobus* showed percentages of inhibition of **more than 50%** at concentrations of **0.0375 mg/ml**, **0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **57.89 ± 0.58%, 72.31 ± 0.24%, 87.19 ± 0.79%, and 92.11 ± 0.94%, respectively**. The IC_{50} value obtained from the dose response curve is 0.0275 mg/ml or 27.5 µg/ml. At that concentration, water extracts of winged beans inhibited 50% of DPPH radicals.

(vi) *Sauropus androgynus*

- Petroleum benzene extracts

As seen in Table 4.7 and illustrated in Figure 4.7, out of the 6 concentrations evaluated the crude petroleum benzene extracts of *S. androgynus* showed percentages of inhibition of **more than 50%** at concentrations of **0.0375 mg/ml**, **0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **50.59 ± 1.02%, 52.45 ± 0.95%, 71.29 ± 0.70%, and 86.30 ± 1.02%, respectively**. The IC_{50} value obtained from the dose response curve is 0.03625 mg/ml or 36.25 µg/ml. At that concentration, petroleum benzene extracts of sweet leaves inhibited 50% of DPPH radicals.

- Chloroform extracts

As seen in Table 4.7 and illustrated in Figure 4.7, the crude chloroform extracts of *S. androgynus* showed percentages of inhibition of **more than 50%** at concentrations of **0.125 mg/ml, 0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **50.50 ± 0.72%, 51.72 ± 0.55%, and 67.49 ± 0.46%, respectively**. The IC_{50} value obtained from the dose response curve is 0.125 mg/ml or 125 µg/ml. At that concentration, chloroform extracts of sweet leaves inhibited 50% of DPPH radicals.

- Methanol extracts

As seen in Table 4.7 and illustrated in Figure 4.7, out of the 6 concentrations evaluated the crude methanol extracts of *S. androgynus* showed percentages of inhibition of **more than 50%** at concentrations of **0.25 mg/ml and 0.5 mg/ml.** At those concentrations, the extract inhibited DPPH radicals by **58.58 ± 0.70%, and 67.66 ± 0.57%, respectively**. The IC_{50} value obtained from the dose response curve is 0.145 mg/ml or 145 µg/ml. At that concentration, methanol extracts of sweet leaves inhibited 50% of DPPH radicals.

- Water extracts

As seen in Table 4.7 and illustrated in Figure 4.7, out of the 6 concentrations evaluated the crude water extracts of *S. androgynus* showed percentages of inhibition of **more than 50%** only at **0.5 mg/ml.** At that concentration, the extract inhibited DPPH radicals by **56.27 ± 0.79%**. The IC_{50} value obtained from the dose response curve is 0.3075 mg/ml or 307.5 µg/ml. At that concentration, water extracts of sweet leaves inhibited 50% of DPPH radicals.

The IC_{50} values of all positive crude extracts are summarized in Table 4.8 and in Figure 4.8. The lower the IC_{50} values, the stronger the radical scavenging activity. Overall, out of the 24 crude extracts tested, the crude petroleum benzene, chloroform and water extracts of curry leaves showed the best DPPH radical inhibition with IC_{50} values of 21.25 µg/ml, 22.5 µg/ml and 32.5 µg/ml.

Table 4.8: The IC_{50} value of the positive crude extracts of selected local vegetables

Local Vegetables	IC_{50} Values (µg/ml)			
	Crude petroleum benzene extract	**Crude chloroform extract**	**Crude methanol extract**	**Crude water extract**
Allium tuberosum	-	467.5	-	192.5
***Apium graveolens* (L.)**	-	-	-	25
***Ipomoea batatas* (L.)**	-	500	292.5	75
***Murraya koenigii* (L.)**	21.25	22.5	-	32.5
Psophocarpus tetragonolobus	400	-	117.5	27.5
***Sauropus androgynus* (L.)**	36.25	125	145	307.5

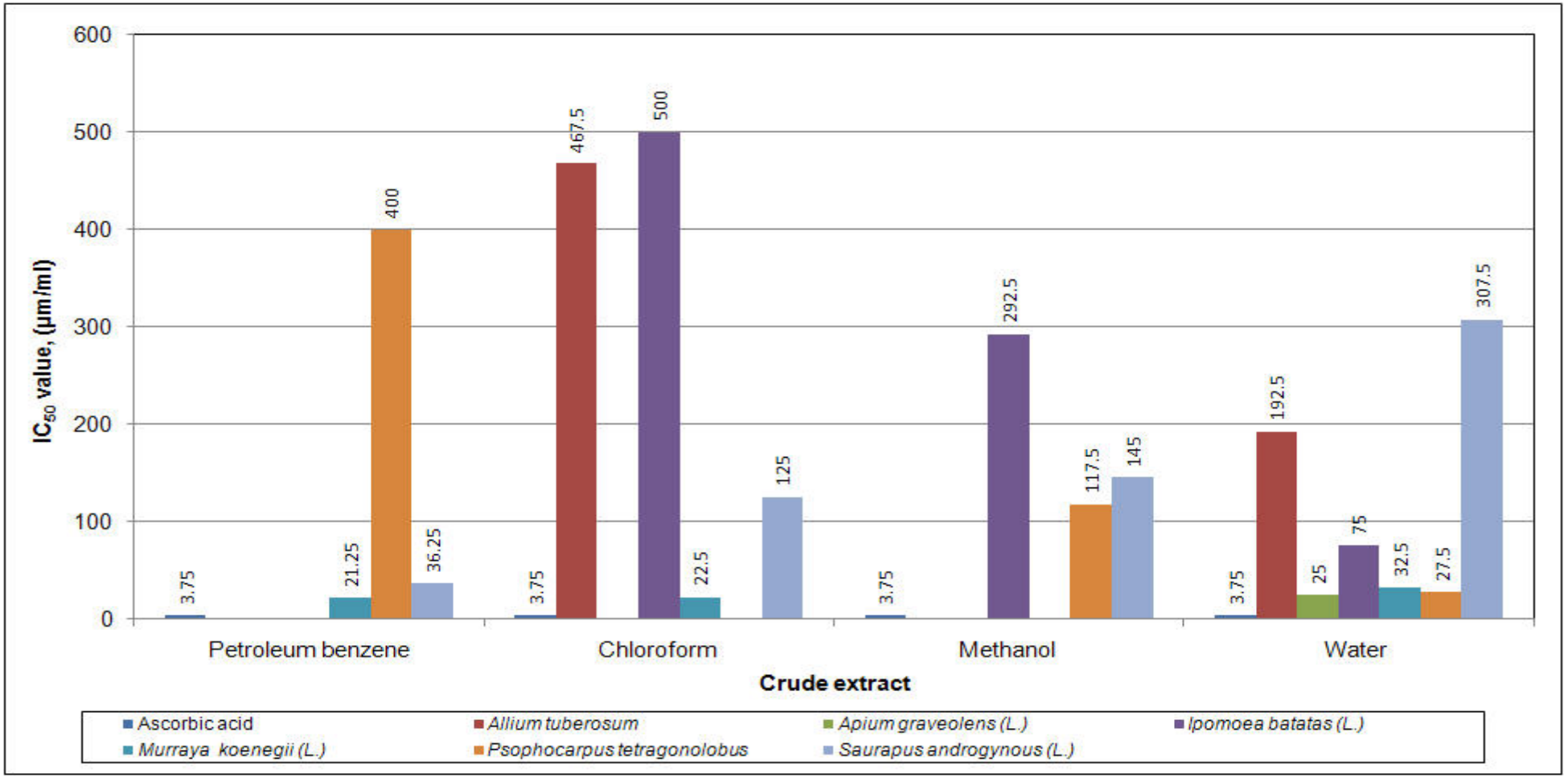


Figure 4.8: Bar chart showing the IC_{50} value of the positive crude extracts of selected local vegetables in comparison with ascorbic acid as positive reference

4.2 Reducing power assay

The potential antioxidant activity of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.)*, Ipomoea batatas* (L.)*, Murraya koenigii* (L.)*, Psophocarpus tetragonolobus, and Sauropus androgynus* were also tested using the reducing power assay at the concentrations of **0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml.** Reducing power assay is a simple assay that gives fast and reproducible results. In this assay, the antioxidant activity was determined on the basis of the ability of the extract to reduce ferric (Fe^{3+}) iron to ferrous (Fe^{2+}) iron. The yellow colour of the test solution was changed into various shades of green and blue colour depending on the reducing power of crude extracts. Butylated hydroxyanisole (BHA) was used as positive reference standard. Optical density (OD) or absorbance of the positive reference and reaction mixtures was quantitated spectrophotometrically at 700 nm.

4.2.1 Reducing power activity of *Allium tuberosum*

The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum* are shown in Table 4.9 and illustrated in Figure 4.9. The reducing powers of these extracts were compared with that of BHA. Increased absorbance of the reaction mixture indicates increased reducing capacity. The reducing capacity of all *Allium tuberosum* crude extracts increased with increasing concentrations.

The reducing capacity of all the crude extracts of *Allium tuberosum* was very low when compared to butylated hydroxyanisole (BHA). Nevertheless, the reducing capacity of methanol extract of garlic chives is slightly higher among the four crude extract tested at the highest concentration tested that is 1 mg/ml.

At the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the absorbance values of BHA at 700 nm was 0.510 ± 0.005, 0.982 ± 0.006, 1.047 ± 0.004, 1.475 ± 0.004 and 2.625 ± 0.004, respectively. At the same concentrations, the absorbance values of crude petroleum ether extracts of *Allium tuberosum* were 0.047 ± 0.001, 0.072 ± 0.002, 0.122 ± 0.002, 0.183 ± 0.001 and 0.243 ± 0.002, respectively; crude chloroform extracts exhibited absorbance values of 0.045 ± 0.003, 0.086 ± 0.002, 0.112 ± 0.002, 0.202 ± 0.001 and 0.225 ± 0.002, respectively, crude methanol extracts exhibited absorbance values of 0.050 ± 0.004, 0.072 ± 0.003, 0.092 ± 0.003, 0.146 ± 0.003 and 0.285 ± 0.003, respectively and crude water extracts exhibited absorbance values of 0.025 ± 0.002, 0.046 ± 0.004, 0.098 ± 0.004, 0.171 ± 0.003, 0.230 ± 0.004, respectively.

Table 4.9: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum* in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract /BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.047±0.001	0.045±0.003	0.050±0.004	0.025±0.002	0.510±0.005
0.125	0.072±0.002	0.086±0.002	0.072±0.003	0.046±0.004	0.982±0.006
0.25	0.122±0.002	0.112±0.002	0.092±0.003	0.098±0.004	1.047±0.004
0.5	0.183±0.001	0.202±0.001	0.146±0.003	0.171±0.003	1.475±0.004
1	0.243±0.002	0.225±0.002	0.285±0.003	0.230±0.004	2.625±0.004

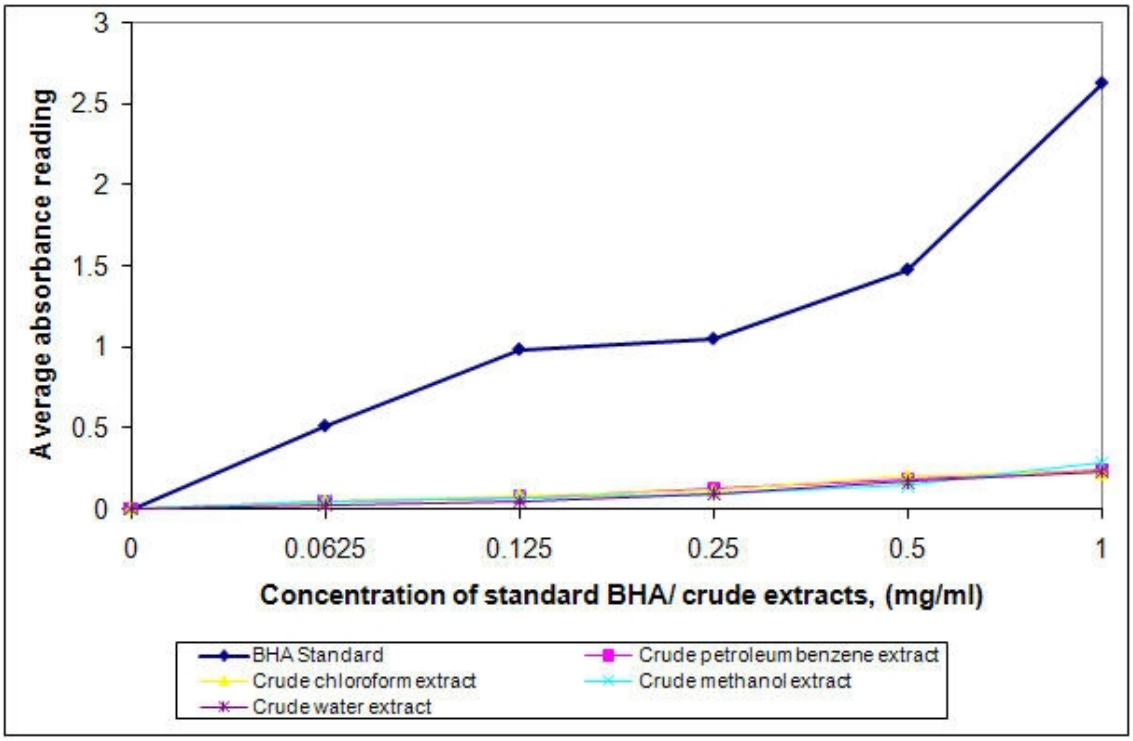


Figure 4.9: Dose-response curves showing reducing activities of *Allium tuberosum* and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.2.2 Reducing power activity of *Apium graveolens* (L.)

The reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) are shown in Table 4.10. As seen in Table 4.10, at the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the absorbance values of BHA at 700 nm was 0.510 ± 0.005, 0.982 ± 0.006, 1.047 ± 0.004, 1.475 ± 0.004 and 2.625 ± 0.004, respectively. At the same concentrations, the absorbance values of crude petroleum ether extracts of *Apium graveolens* (L.) were 0.046 ± 0.003, 0.062 ± 0.003, 0.097 ± 0.002, 0.183 ± 0.004 and 0.295 ± 0.003, respectively; crude chloroform extracts exhibited absorbance values of 0.057 ± 0.002, 0.092 ± 0.003, 0.151 ± 0.004, 0.212 ± 0.003 and 0.288 ± 0.002, respectively, crude methanol extracts exhibited absorbance values of 0.099 ± 0.001, 0.155 ± 0.003, 0.354 ± 0.002, 0.552 ± 0.001 and 0.696 ± 0.001, respectively and crude

water extracts exhibited absorbance values of 0.055 ± 0.004, 0.084 ± 0.004, 0.144 ± 0.004, 0.213 ± 0.003, 0.322 ± 0.002, respectively.

Table 4.10: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract/BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.046±0.003	0.057±0.002	0.099±0.001	0.055±0.004	0.510±0.005
0.125	0.062±0.003	0.092±0.003	0.155±0.003	0.084±0.004	0.982±0.006
0.25	0.097±0.002	0.151±0.004	0.354±0.002	0.144±0.004	1.047±0.004
0.5	0.183±0.004	0.212±0.003	0.552±0.001	0.213±0.003	1.475±0.004
1	0.295±0.003	0.288±0.002	0.696±0.001	0.322±0.002	2.625±0.004

Figure 4.10 illustrates the dose-response curves of the reducing power of BHA, crude petroleum benzene, chloroform and methanol extracts of *Apium graveolens* (L.). As seen, the reducing capacity of *Apium graveolens* (L.) was much lower than that of BHA. In general, the reducing power increased with increasing concentrations of crude extracts and among all extracts of *Apium graveolens* (L.) the crude methanol extract showed the highest reducing power capacity at 1 mg/ml.

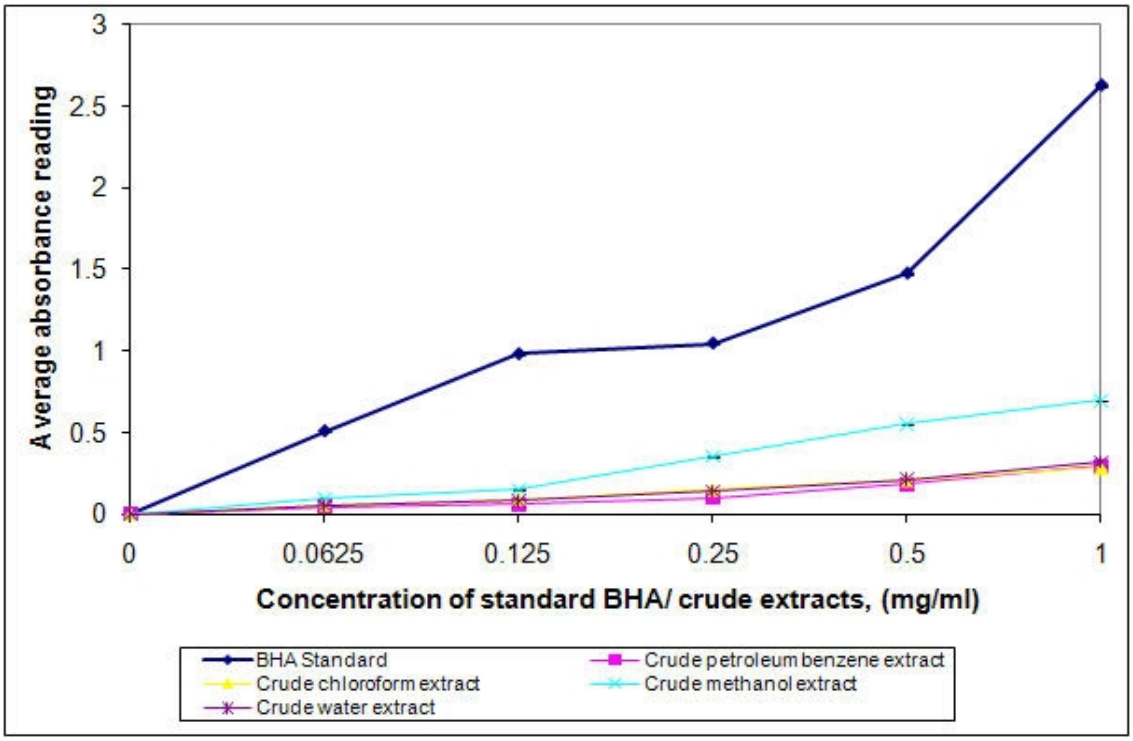


Figure 4.10: Dose-response curves showing reducing activities of *Apium graveolens* (L.) and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.2.3 Reducing power activity of *Ipomoea batatas* (L.)

The reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum* are shown in Table 4.11 and illustrated in Figure 4.11. Increased absorbance of the reaction mixture at 700 nm indicates increased reducing capacity. The reducing capacity all crude extracts of *Ipomoea batatas* (L.) increased with increasing concentrations.

As seen in Table 4.11, at the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the absorbance values of BHA at 700 nm was 0.510 ± 0.005, 0.982 ± 0.006, 1.047 ± 0.004, 1.475 ± 0.004 and 2.625 ± 0.004, respectively. At the same concentrations, the absorbance values of crude petroleum ether extracts of *Apium graveolens* (L.) were 0.119 ± 0.002, 0.153 ± 0.002, 0.292 ± 0.001, 0.344 ± 0.002 and 0.493 ± 0.005, respectively; crude chloroform extracts exhibited absorbance values of 0.120 ± 0.001, 0.180 ± 0.002, 0.270 ± 0.002, 0.356 ± 0.003 and 0.481 ± 0.001,

respectively, crude methanol extracts exhibited absorbance values of 0.094 ± 0.004, 0.143 ± 0.004, 0.199 ± 0.002, 0.282 ± 0.004 and 0.432 ± 0.004, respectively and crude water extracts exhibited absorbance values of 0.126 ± 0.002, 0.227 ± 0.002, 0.343 ± 0.003, 0.491 ± 0.002, 0.686 ± 0.003, respectively. Crude water extract showed the highest reducing power capacity (0.686 ± 0.003) while crude petroleum benzene, chloroform and methanol showed lower and almost similar reducing capacities of 0.493 ± 0.005, 0.481 ± 0.001 and 0.432 ± 0.004, respectively at 1 mg/ml. Thus, all crude extract of sweet potato leaves showed moderate reducing capacities.

Table 4.11: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Ipomoea batatas* (L.) in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract/BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.119±0.002	0.120±0.001	0.094±0.004	0.126±0.002	0.510±0.005
0.125	0.153±0.002	0.180±0.002	0.143±0.004	0.227±0.002	0.982±0.006
0.25	0.292±0.001	0.270±0.002	0.199±0.002	0.343±0.003	1.047±0.004
0.5	0.344±0.002	0.356±0.003	0.282±0.004	0.491±0.002	1.475 ±0.004
1	0.493±0.005	0.481±0.001	0.432±0.004	0.686±0.003	2.625±0.004

The dose-curves (Figure 4.11) showed that the reducing power of crude extracts was increased with increasing concentrations. The absorbance values of crude extracts showed lower absorbance value thus weaker reducing power than that of standard references (BHA) at all concentrations ranging from 0.0625 mg/ml to 1 mg/ml.

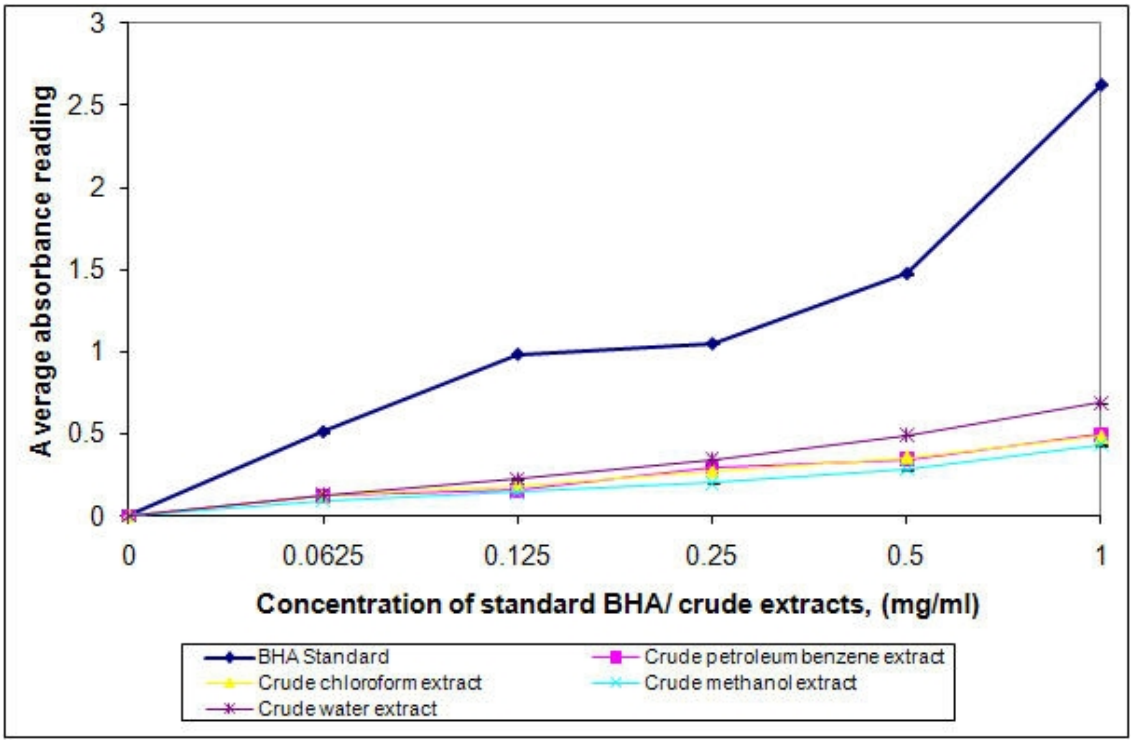


Figure 4.11: Dose-response curves showing reducing activities of *Ipomoea batatas* (L.) and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.2.4 Reducing power activity of *Murraya koenigii* (L.)

The reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) are shown in Table 4.12. As seen in Table 4.12, crude chloroform extract showed very good reducing power capacity of 0.415 ± 0.002, 0.763 ± 0.001, 1.134, ± 0.004, 1.623 ± 0.003 and 1.833 ± 0.003 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. This is followed by crude petroleum benzene which also showed satisfactory reducing power capacity of 0.254 ± 0.004, 0.468 ± 0.001, 0.637 ± 0.002, 1.003 ± 0.001 and 1.208 ± 0.006 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. Crude water extract showed very low reducing power capacity of 0.076 ± 0.002, 0.095 ± 0.003, 0.132 ± 0.003, 0.202 ± 0.003, and 0.235 ± 0.003 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. Crude methanol extract showed lowest reducing power capacity of 0.039 ± 0.001, 0.076 ± 0.003, 0.097 ± 0.001, 0.125 ± 0.002 and 0.192

± 0.002 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively.

Table 4.12: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract/BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.254±0.004	0.415±0.002	0.039±0.001	0.076±0.002	0.510±0.005
0.125	0.468±0.001	0.763±0.001	0.076±0.003	0.095±0.003	0.982±0.006
0.25	0.637±0.002	1.134±0.004	0.097±0.001	0.132±0.003	1.047±0.004
0.5	1.003±0.001	1.623±0.003	0.125±0.001	0.202±0.003	1.475±0.004
1	1.208±0.006	1.833±0.003	0.192±0.002	0.235±0.003	2.625±0.004

The dose-response curves (Figure 4.12) showed that the reducing power of crude extracts was increased with increasing concentrations. When compared with those of BHA, crude chloroform extracts showed good reducing activity.while petroleum benzene extracts of curry leaves showed moderate reducing activity. In comparison, the reducing capacities of crude water and methanol extracts were much weaker.

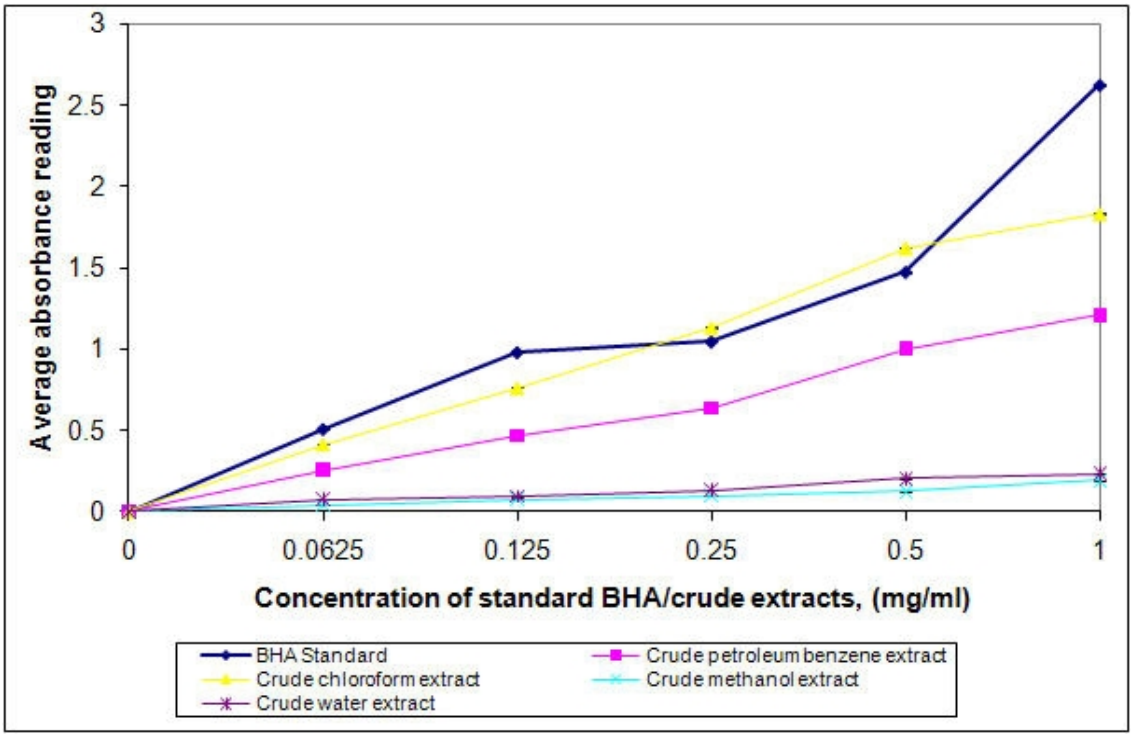


Figure 4.12: Dose-response curves showing reducing activities of *Murraya koenigii* (L.) and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.2.5 Reducing power activity of *Psophocarpus tetragonolobus*

The reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* are shown in Table 4.13 and illustrated in Figure 4.13. Increased absorbance of the reaction mixture indicates increased reducing capacity.

As seen in Table 4.13, at the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the absorbance values of BHA at 700 nm was 0.510 ± 0.005, 0.982 ± 0.006, 1.047 ± 0.004, 1.475 ± 0.004 and 2.625 ± 0.004, respectively. At the same concentrations, the absorbance values of crude petroleum ether extracts of *Psophocarpus tetragonolobus* were 0.051 ± 0.002, 0.102 ± 0.002, 0.183 ± 0.003, 0.214 ± 0.001 and 0.272 ± 0.002, respectively; crude chloroform extracts exhibited absorbance values of 0.065 ± 0.003, 0.085 ± 0.002, 0.105 ± 0.002, 0.160 ± 0.003 and 0.223 ± 0.002, respectively, crude methanol extracts exhibited absorbance values of

0.103 ± 0.003, 0.187 ± 0.002, 0.244 ± 0.002, 0.301 ± 0.002 and 0.482 ± 0.003, respectively and crude water extracts exhibited absorbance values of 0.046 ± 0.003, 0.074 ± 0.005, 0.114 ± 0.004, 0.161 ± 0.003, 0.282 ± 0.002, respectively. Crude methanol extract showed the highest reducing power capacity (0.482 ± 0.003) while crude petroleum benzene, chloroform and water showed lower and almost similar reducing capacities of 0.272 ± 0.002 , 0.223 ± 0.002 and 0.282 ± 0.002, respectively at 1 mg/ml.

Table 4.13: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract/BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.051±0.002	0.065±0.003	0.103±0.003	0.046±0.003	0.510±0.005
0.125	0.102±0.002	0.085±0.002	0.187±0.002	0.074±0.005	0.982±0.006
0.25	0.183±0.003	0.105±0.002	0.244±0.002	0.114±0.004	1.047±0.004
0.5	0.214±0.001	0.160±0.003	0.301±0.002	0.161±0.003	1.475±0.004
1	0.272±0.002	0.223±0.002	0.482±0.003	0.282±0.002	2.625±0.004

Figure 4.13 illustrates the dose-response curves of the reducing power of BHA, crude petroleum benzene, chloroform and methanol extracts of *Psophocarpus tetragonolobus.* As seen, the reducing capacity of *Psophocarpus tetragonolobus* extracts was weak and was much lower than that of BHA. In general, the reducing power increased slightly with increasing concentrations of crude extracts.

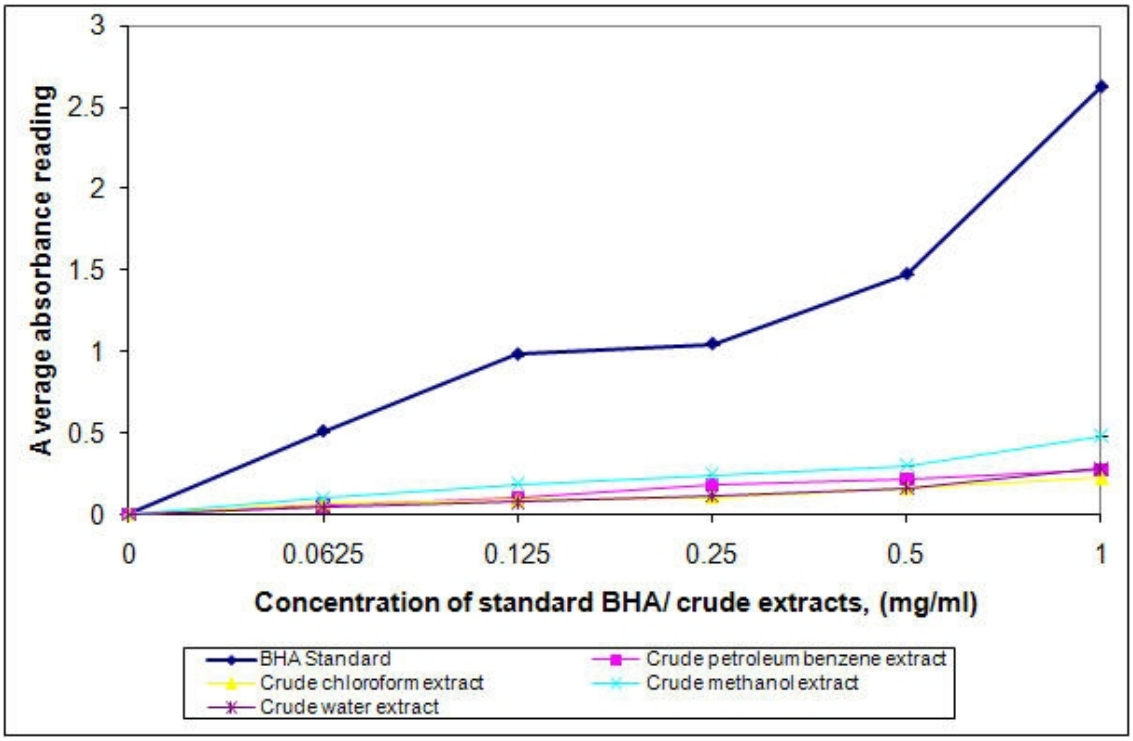


Figure 4.13: Dose-response curves showing reducing activities of *Psophocarpus tetragonolobus* and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.2.6 Reducing power activity of *Sauropus androgynus*

The reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus* are shown in Table 4.14. As seen in Table 4.14, when compared to that of BHA, crude water extract showed good reducing power capacity of 0.336 ± 0.005, 0.595 ± 0.003, 0.677 ± 0.003, 1.038 ± 0.003 and 1.529 ± 0.004 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. This is followed by crude petroleum benzene extract which showed less than moderate reducing power capacity of 0.170 ± 0.002, 0.270 ± 0.001, 0.327 ± 0.003, 0.545 ± 0.003 and 0.656 ± 0.004 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. Crude methanol extract showed weak reducing power of 0.098 ± 0.003, 0.183 ± 0.005, 0.242 ± 0.002, 0.311 ± 0.001 and 0.443 ± 0.002 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. Crude chloroform extract showed lowest reducing capacities of 0.051 ± 0.003, 0.077 ± 0.002, 0.105 ± 0.003,

0.135 ± 0.002 and 0.232 ± 0.002 at 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively.

Table 4.14: The reducing power of crude petroleum ether, chloroform, methanol and water extracts of *Sauropus androgynus* in comparison with Butylated hydroxyanisole (BHA)

Concentration of crude extract/BHA (mg/ml)	Average Absorbance Reading at 700 nm				
	Petroleum Benzene	Chloroform	Methanol	Water	Butylated hydroxyanisole (BHA)
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	0.170±0.002	0.051±0.003	0.098±0.003	0.336±0.005	0.510±0.005
0.125	0.270±0.001	0.077±0.002	0.183±0.005	0.595±0.003	0.982±0.006
0.25	0.327±0.003	0.105±0.003	0.242±0.002	0.677±0.003	1.047±0.004
0.5	0.545±0.003	0.135±0.002	0.311±0.001	1.038±0.004	1.475±0.004
1	0.656±0.004	0.232±0.002	0.443±0.002	1.529±0.004	2.625±0.004

Figure 4.14 illustrates the dose-response curves of the reducing power of BHA, crude petroleum benzene, chloroform and methanol extracts of *Sauropus androgynus***.**. As seen, water extracts of *Sauropus androgynus* showed good reducing activity when compared to that of BHA. In general, the reducing power increased slightly with increasing concentrations of crude extracts.

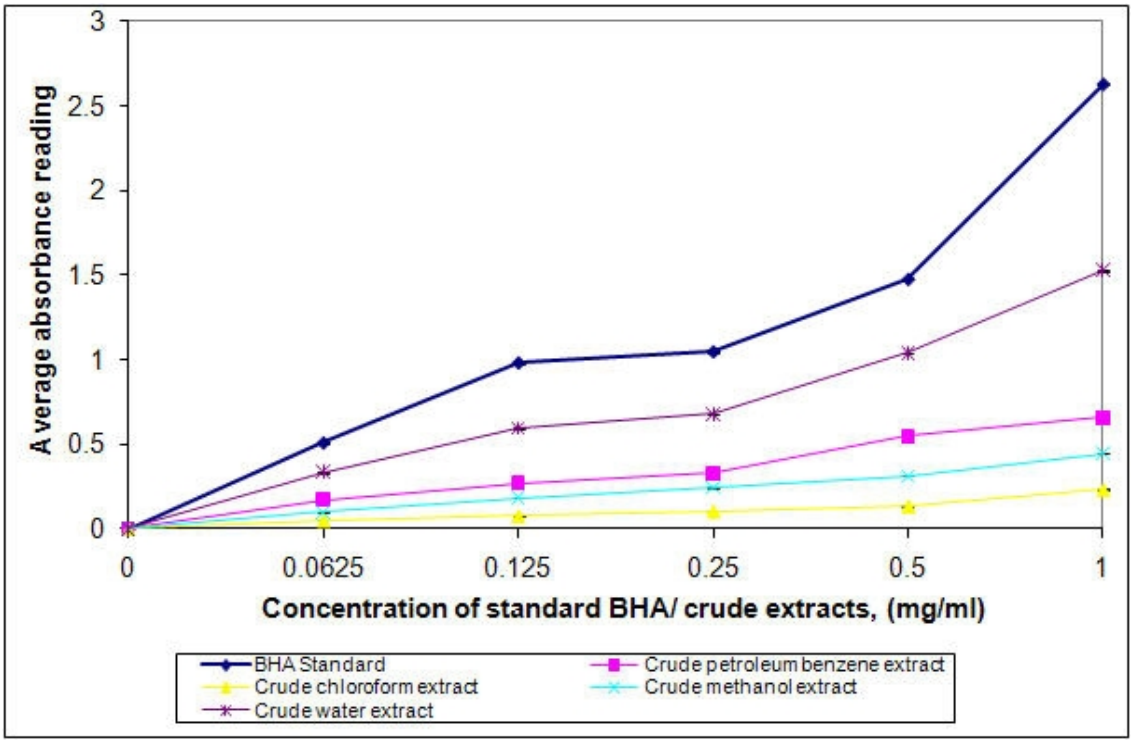


Figure 4.14: Dose-response curves showing reducing activities of *Sauropus androgynus* and butylated hydroxyanisole (BHA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

The reducing potential of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* are shown in Table 4.15 at the highest concentration tested (1 mg/ml) in descending order. The higher the reducing capacities of the crude extracts, the stronger the ability of the extracts to reduce ferric (Fe^{3+}) iron to ferrous (Fe^{2+}) iron.

Table 4.15: The reducing power of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* tested at 1 mg/ml

Absorbance Reading at 1 mg/ml	Crude Extract of Selected Vegetables
1.833 ± 0.003	Curry leaves + chloroform
1.529 ± 0.004	Sweet leaves + water
1.208 ± 0.006	Curry leaves + petroleum benzene
0.696 ± 0.001	Celeries + methanol
0.686 ± 0.003	Sweet potato leaves + water
0.656 ± 0.004	Sweet leaves + petroleum benzene
0.493 ± 0.005	Sweet potato leaves + petroleum benzene
0.482 ± 0.003	Winged beans + methanol
0.481 ± 0.001	Sweet potato leaves + chloroform
0.443 ± 0.002	Sweet leaves + methanol
0.432 ± 0.004	Sweet potato leaves + methanol
0.322 ± 0.002	Celeries + water
0.295 ± 0.003	Celeries +petroleum benzene
0.288 ± 0.002	Celeries + chloroform
0.285 ± 0.003	Garlic chives + methanol
0.282 ± 0.002	Winged beans + water
0.272 ± 0.002	Winged beans + petroleum benzene
0.243 ± 0.002	Garlic chives + petroleum benzene
0.235 ± 0.003	Curry leaves + water
0.232 ± 0.002	Sweet leaves + chloroform
0.230 ± 0.004	Garlic chives + water
0.225 ± 0.002	Garlic chives +chloroform
0.223 ± 0.002	Winged beans + chloroform
0.192 ± 0.002	Curry leaves + methanol

As shown in the table, chloroform extracts of curry leaves showed the highest reducing activity while methanol extract of curry leaves showed the lowest reducing activity. Water extract of sweet leaves and petroleum benzene extract of curry leaves on the other hand showed moderate reducing activity.

4.3 Metal chelating assay

Metal chelating assay evaluates the ability of test extracts to chelate ferrous ion and prevent the formation of ferrozine-Fe^{2+} complex. The metal chelating activity of crude extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* were evaluated at different concentration ranging from **0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml**. EDTA is a strong metal chelator, hence it was used as positive standard reference as metal chelator agent in this metal chelating assay.

4.3.1 The metal chelating activity of *Allium tuberosum*

Metal chelating activities of crude petroleum benzene, chloroform, methanol, and water extracts of *Allium tuberosum* and standard reference EDTA are shown in Table 4.16. As seen in Table 4.16, at the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26% , 95.65 ± 0.08%, 96.59± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Allium tuberosum* were 30.58 ± 0.25%, 43.10 ± 0.18%, 61.03 ± 0.48%, 71.24 ± 0.14% and 81.09 ± 0.27%, respectively; crude chloroform extracts exhibited chelating effects of 28.14 ± 0.10%, 34.72 ± 0.42%, 47.49 ± 0.13%, 59.81 ± 0.22% and 78.05 ± 0.25%, respectively, crude methanol extracts exhibited chelating effect of 18.03 ± 0.26%, 31.04 ± 0.32%, 54.32 ± 0.39%, 63.02 ± 0.23% and 70.68 ± 0.22%, respectively and crude water extracts exhibited chelating effect of 14.08 ± 0.15%, 21.81 ± 0.02%, 29.33 ± 0.25%, 42.89 ± 0.08%, 52.23 ± 0.03%, respectively.

As seen, crude petroleum benzene, chloroform and methanol extracts of *Allium tuberosum* showed stronger metal chelating activity as compared to the crude water extracts. At the concentrations of 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml the crude

petroleum benzene extracts exhibited percentage of inhibition of more than 50% at being 61.03%, 71.24% and 81.09%, respectively. On the other hand, at the same concentration, metal chelating capacity of crude methanol extracts were found to be 54.32%, 63.02% and 70.68%, respectively. Crude chloroform extract showed 59.81% and 78.05% at 0.5 mg/ml and 1 mg/ml, respectively. The chelating effect on ferrous ion was much weaker by 52.23% in crude water extract which only exhibit more than 50% of inhibition at 1 mg/ml. EDTA exhibited percentage of inhibition of more than 50% at all concentration with 94.39% at 0.0625 mg/ml, 95.65% at 0.125 mg/ml, 96.59% at 0.25 mg/ml, 98.10% at 0.5 mg/ml and 98.63% at 1 mg/ml. It is indeed a strong metal chelator.

Table 4.16: Metal chelating activity of crude petroleum benzene, chloroform and methanol extracts of *Allium tuberosum* and standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Garlic Chives/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	30.58±0.25	28.14±0.10	18.03±0.26	14.08±0.15	94.39±0.26
0.125	43.10±0.18	34.72±0.42	31.04±0.32	21.81±0.02	95.65±0.08
0.25	61.03±0.48	47.49±0.13	54.32±0.39	29.33±0.25	96.59±0.20
0.5	71.24±0.14	59.81±0.22	63.02±0.23	42.89±0.08	98.10±0.08
1	81.09±0.27	78.05±0.25	70.68±0.22	52.23±0.03	98.63±0.13

Dose-response curves illustrating the metal chelating activities of EDTA, crude petroleum benzene, chloroform and methanol extracts of *Allium tuberosum* are shown in Figure 4.15. As seen, the metal chelating activities of crude petroleum benzene, chloroform, methanol and water extracts were low at low concentration of 0.0625 mg/ml. Crude petroleum benzene, chloroform and methanol extracts showed good chelating activities when compared with EDTA at the concentration of 1mg/ml. Crude water extract only showed moderare chelating activity when compared with EDTA at 1 mg/ml. The metal chelating activities of all crude extracts of garlic chives increased gradually with increasing concentrations ranging from 0.0625 mg/ml to 1 mg/ml.

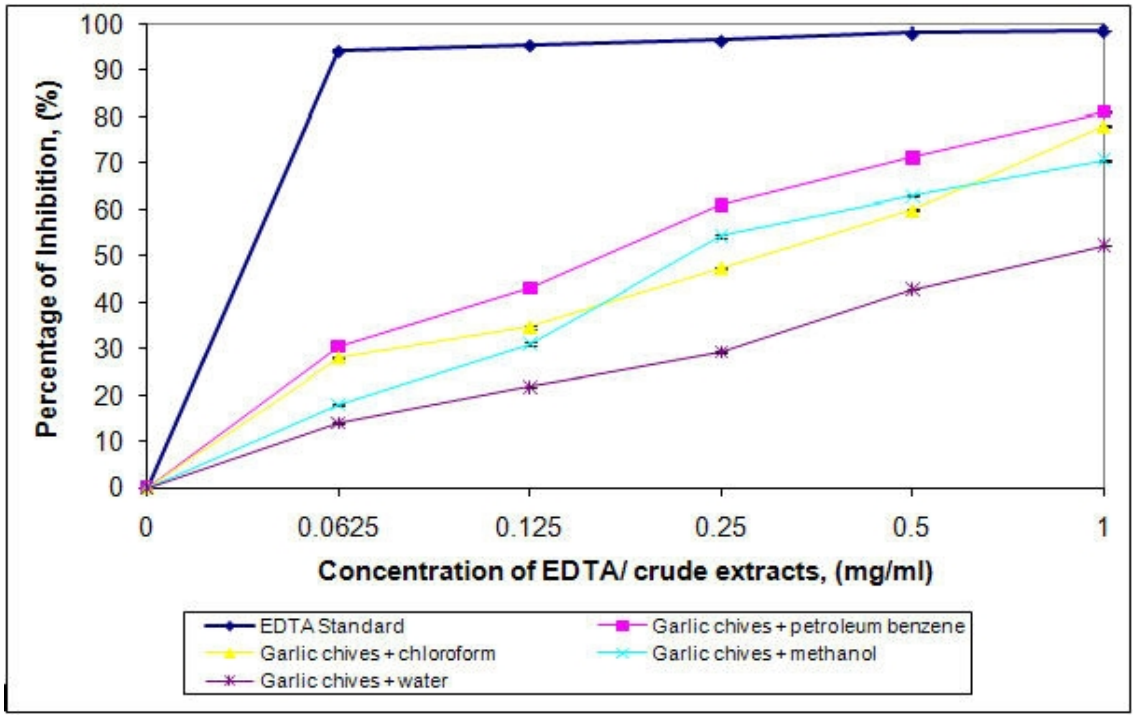


Figure 4.15: Dose-response curves showing chelating activities of *Allium tuberosum* and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.3.2 The metal chelating activity of *Apium graveolens* (L.)

Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) and standard reference EDTA are shown in Table 4.17. At the concentrations of 0.0625 mg/ml, 0.12 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26%, 95.65 ± 0.08%, 96.59 ± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Apium graveolens* (L.) were 49.03 ± 0.34%, 61.01 ± 0.17%, 71.05 ± 0.12%, 77.91± 0.18% and 81.02 ± 0.14%, respectively; crude chloroform extracts exhibited chelating effects of 37.69 ± 0.09%, 48.33 ± 0.28%, 56.42 ± 0.15%, 60.77 ± 0.20% and 73.45 ± 0.17%, respectively, crude methanol extracts exhibited chelating effect of 16.95 ± 0.34%, 30.17 ± 0.23%, 41.10 ± 0.41%, 50.70 ± 0.28% and 59.52 ± 0.34%, respectively and crude water extracts

exhibited chelating effect of 48.84 ± 0.44%, 60.10 ± 0.23%, 68.81 ± 0.23%, 73.02 ± 0.18%, 87.46 ± 0.28%, respectively.

As seen, among the four extract, the highest percentage of metal chelating capacity of *Apium graveolens* (L.) was for the crude water extract following by crude petroleum benzene, chloroform and methanol. They exhibited 87.46%, 81.02%, 73.45% and 59.52% chelation of ferrous ion at 1 mg/ml.

Table 4.17: Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) and standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Celery/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	49.03±0.34	37.69±0.09	16.95±0.34	48.84±0.44	94.39±0.26
0.125	61.01±0.17	48.33±0.28	30.17±0.23	60.10±0.23	95.65±0.08
0.25	71.05±0.12	56.42±0.15	41.10±0.41	68.81±0.23	96.59±0.20
0.5	77.91±0.18	60.77±0.20	50.70±0.28	73.02±0.18	98.10±0.08
1	81.02±0.14	73.45±0.17	59.52±0.34	87.46±0.28	98.63±0.13

Dose-response curves of crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) with standard reference EDTA are shown in Figure 4.16. As seen, the metal chelating capacities on ferrous ion for both petroleum benzene and water crude extracts was quite high, although much lower than EDTA. Both the crude extracts exhibit more than 50% metal chelating capacity from the concentration of 0.125 mg/ml. The metal chelating capacity on ferrous ion for crude chloroform and methanol was at satisfactory level too as they exhibit more than 50% metal chelating capacity. The metal chelating activities of crude petroleum benzene, chloroform, methanol and water extracts were moderate, moderate low and low, respectively at concentration of 0.0625 mg/ml. The metal chelating activities of crude petroleum benzene and chloroform however were good when compared with EDTA at

higher concentration of 1 mg/ml while crude methanol extract only showed moderate chelating activity when compared with EDTA at 1 mg/ml.

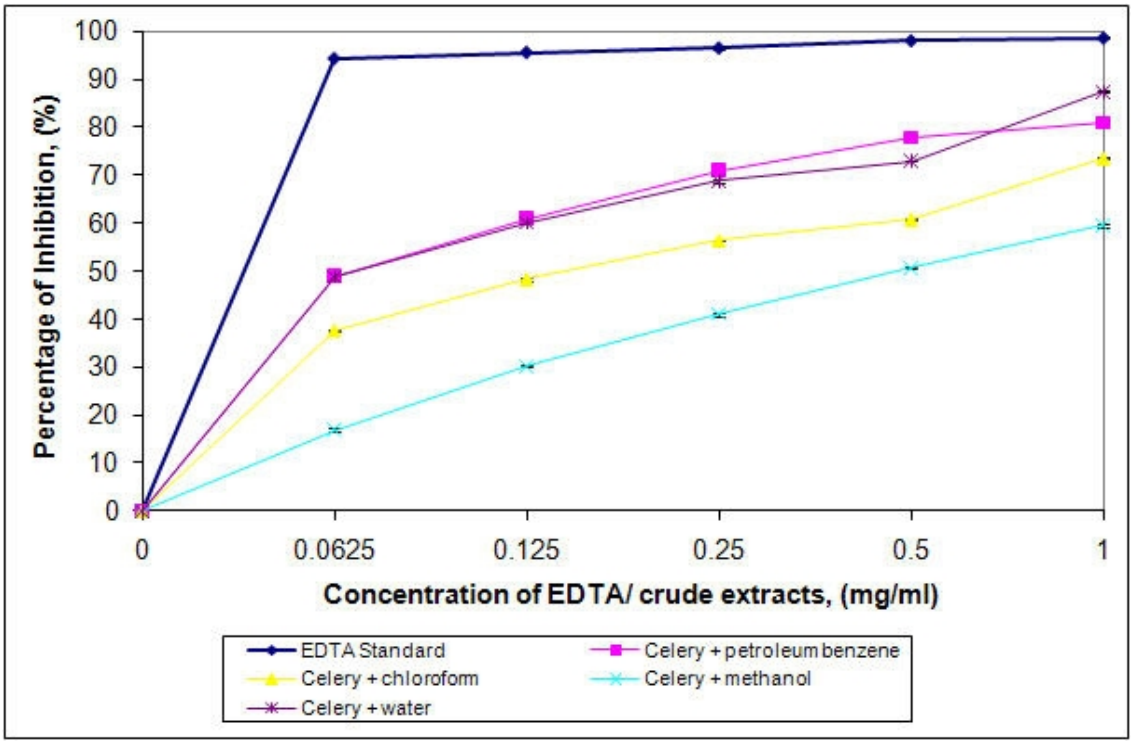


Figure 4.16: Dose-response curves showing chelating activities of *Apium graveolens* (L.) and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.3.3 The metal chelating activity of *Ipomoea batatas* (L.)

Metal chelating activity of crude petroleum benzene, chloroform, methanol, and water extracts of *Ipomoea batatas* (L.) and standard reference EDTA are shown in Table 4.18. At the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26%, 95.65 ± 0.08 %, 96.59 ± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Ipomoea batatas* (L.) were 36.14 ± 0.19%, 48.83 ± 0.27%, 64.61 ± 0.17%, 69.81 ± 0.09% and 79.40 ± 0.14%, respectively; crude chloroform extracts exhibited chelating effects of 40.68 ± 0.30%, 53.74 ± 0.39%, 60.15 ± 0.23%, 77.06 ± 0.18% and 88.56 ± 0.19%, respectively, crude methanol extracts exhibited chelating effect of 9.42± 0.16%, 23.89 ± 0.14%, 30.22 ±

0.18%, 38.62 ± 0.16% and 46.03 ± 0.30%, respectively and crude water extracts exhibited chelating effect of 15.42 ± 0.34%, 28.92 ± 0.17%, 37.33 ± 0.18%, 49.02 ± 0.32%, 56.14 ± 0.34%, respectively.

As seen, crude chloroform extracts from *Ipomoea batatas* (L.) showed stronger metal chelating activity as compared to the crude petroleum benzene and water extracts. The crude chloroform extracts exhibited percentage of inhibition of more than 50 % at 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, respectively. On the other hand, metal chelating capacity for 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml of crude petroleum benzene extracts were found to be more than 50%. The chelating effect on ferrous ion was weaker in crude water extracts which only exhibit more than 50% of inhibition at 1 mg/ml. However, the metal chelating capacity of crude methanol extract was very low as it did not exhibit more than 50% of inhibition at any concentration tested.

Table 4.18: Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Ipomoea batatas* (L.) and with standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Sweet Potato Leaves/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	36.14±0.19	40.68±0.30	9.42±0.16	15.42±0.34	94.39±0.26
0.125	48.83±0.27	53.74±0.39	23.89±0.14	28.92±0.17	95.65±0.08
0.25	64.61±0.17	60.15±0.23	30.22±0.18	37.33±0.18	96.59±0.20
0.5	69.81±0.09	77.06±0.18	38.62±0.16	49.02±0.32	98.10±0.08
1	79.40±0.14	88.56±0.19	46.03±0.30	56.14±0.34	98.63±0.13

Dose-response curves of EDTA and crude petroleum benzene, chloroform, methanol and water extracts of *Ipomoea batatas* (L.) with standard reference EDTA are shown in Figure 4.17. As seen, the metal chelating capacities on ferrous ion for both chloroform and petroleum benzene crude extracts was high, when compared with EDTA. Crude water extract exhibited moderate metal chelating activity. However, the metal chelating capacity on ferrous ion for crude methanol extract was moderate low.

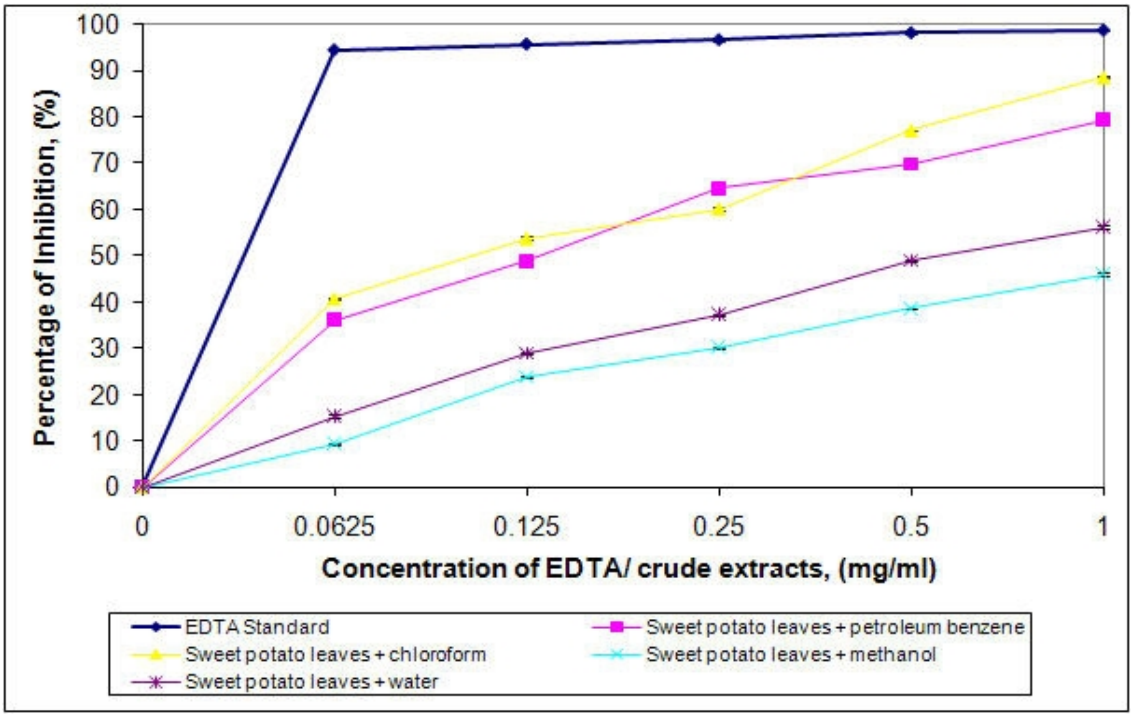


Figure 4.17: Dose-response curves showing chelating activities of *Ipomoea batatas* (L.) and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.3.4 The metal chelating activity of *Murraya koenigii* (L.)

Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) and standard reference EDTA are shown in Table 4.19. At the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26%, 95.65 ± 0.08%, 96.59 ± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Murraya koenigii* (L.) were 24.83 ± 0.26%, 46.01 ± 0.09%, 53.86 ± 0.19%, 59.78 ± 0.25% and 73.61 ± 0.22%, respectively; crude chloroform extracts exhibited chelating effects of 23.25 ± 0.47%, 38.79 ± 0.52%, 55.57 ± 0.45%, 65.37 ± 0.33% and 78.30 ± 0.23%, respectively, crude methanol extracts exhibited chelating effect of 30.26 ± 0.22%, 50.14 ± 0.24%, 64.25 ± 0.90%, 78.29 ± 0.27% and 88.60 ± 0.20%, respectively and crude water extracts

exhibited chelating effect of 9.84 ± 0.26%, 24.59 ± 0.16%, 46.68 ± 0.78%, 57.67 ± 0.23%, 66.81 ± 0.26%, respectively.

As seen, among the four extract, the highest percentage of metal chelating capacity of *Murraya koenigii* (L.) was for the crude methanol extract followed by crude chloroform, petroleum benzene and methanol. They exhibited 88.60%, 78.30%, 73.61% and 66.81% chelation of ferrous ion at 1 mg/ml. All crude extracts of *Murraya koenigii* (L.) exhibit more than 50% metal chelating capacity and thus considered as good metal chelators.

Table 4.19: Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) and with standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Curry Leaves/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
CONTROL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	24.83±0.26	23.25±0.47	30.26±0.22	9.84±0.26	94.39±0.26
0.125	46.01±0.09	38.79±0.52	50.14±0.24	24.59±0.16	95.65±0.08
0.25	53.86±0.19	55.57±0.45	64.25±0.90	46.68±0.78	96.59±0.20
0.5	59.78±0.25	65.37±0.33	78.29±0.27	57.67±0.23	98.10±0.08
1	73.61±0.22	78.30±0.23	88.60±0.20	66.81±0.26	98.63±0.13

Dose-response curves of crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) with standard reference EDTA are shown in Figure 4.18. As seen, the metal chelating capacities on ferrous ion for methanol, petroleum benzene, chloroform and water crude extracts was low at low concentration of 0.0625 mg/ml. However, at 1mg/ml crude methanol, chloroform, petroleum benzene and water showed good metal chelating capacities.

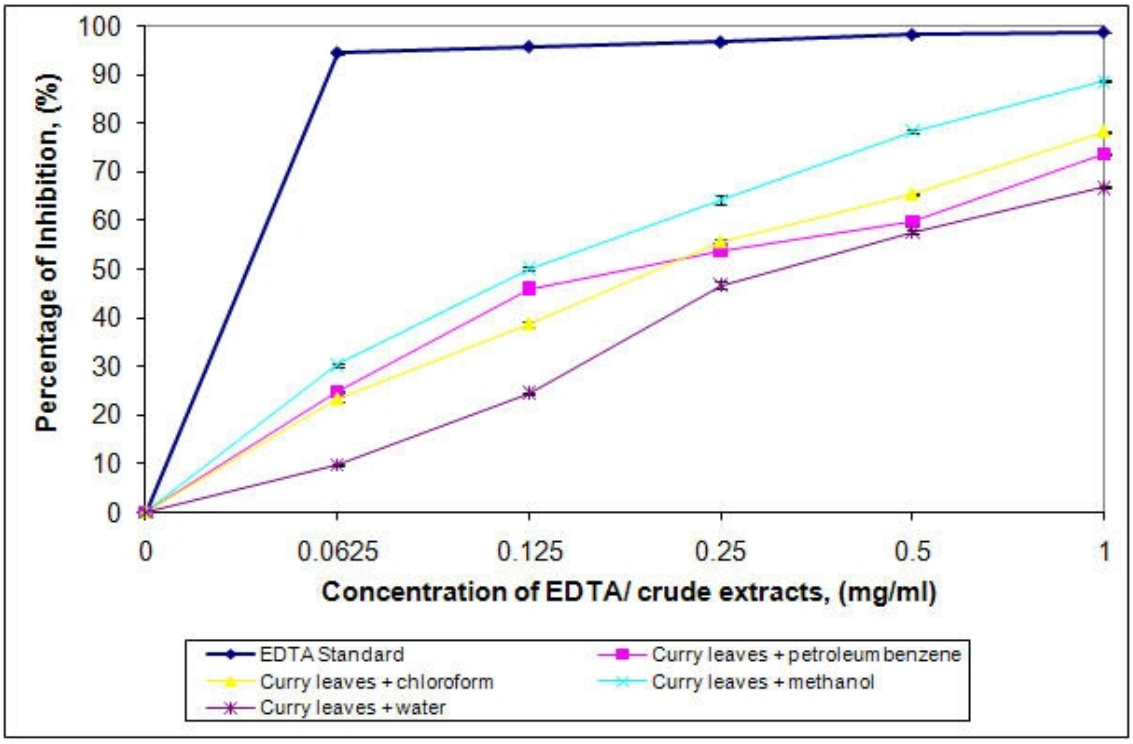


Figure 4.18: Dose-response curves showing chelating activities of *Murraya koenigii* (L.) and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.3.5 The metal chelating activity of *Psophocarpus tetragonolobus*

Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* and standard reference EDTA are shown in Table 4.20. At the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26%, 95.65 ± 0.08%, 96.59 ± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Psophocarpus tetragonolobus* were 26.43 ± 0.33%, 41.29 ± 0.13%, 57.50 ± 0.21%, 63.79 ± 0.23% and 77.02 ± 0.14%, respectively; crude chloroform extracts exhibited chelating effects of 54.25 ± 0.22%, 61.34 ± 0.23%, 67.34 ± 0.25%, 72.04 ± 0.29% and 79.78 ± 0.13%, respectively, crude methanol extracts exhibited chelating effect of 24.58 ± 0.34%, 31.50 ± 0.09%, 40.65± 0.09%, 50.22 ± 0.26% and 58.94 ± 0.26%,

respectively and crude water extracts exhibited chelating effect of 30.96 ± 0.02%, 41.97 ± 0.24%, 52.95 ± 0.17%, 60.81 ± 0.18%, 67.02 ± 0.33%, respectively.

As seen, among the four extract, the highest percentage of metal chelating capacity of *Psophocarpus tetragonolobus* was for the crude chloroform extract followed by crude petroleum benzene, water and methanol. They exhibited 79.78%, 77.02%, 67.02% and 58.94% chelation of ferrous ion at 1 mg/ml.

Table 4.20: Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* and with standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Winged Bean/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	26.43±0.33	54.25±0.22	24.58±0.34	30.96±0.02	94.39±0.26
0.125	41.29±0.13	61.34±0.23	31.50±0.09	41.97±0.24	95.65±0.08
0.25	57.50±0.21	67.34±0.25	40.65±0.09	52.95±0.17	96.59±0.20
0.5	63.79±0.23	72.04±0.29	50.22±0.26	60.81±0.18	98.10±0.08
1	77.02±0.14	79.78±0.13	58.94±0.26	67.02±0.33	98.63±0.13

Dose-response curves of crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* and standard reference EDTA are shown in Figure 4.19. As seen, the metal chelating capacities on ferrous ion for chloroform and petroleum benzene crude extracts were good when compared to that of EDTA at 1 mg/ml. Crude water extract exhibit moderate high metal chelating capacity whereas crude methanol extract exhibited moderate metal chelating capacity when compared to that of EDTA at 1 mg/ml. In general, all the crude extracts exhibit more than 50% metal chelating capacity atleast at the concentration of 0.5 mg/ml.

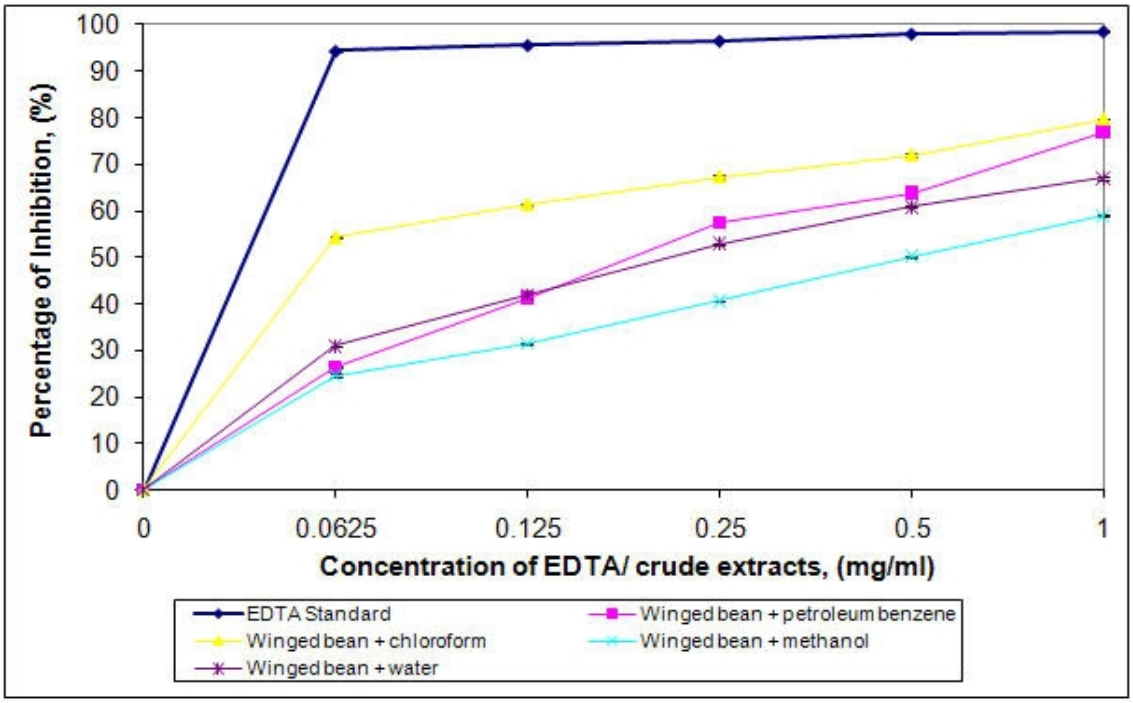


Figure 4.19: Dose-response curves showing chelating activities of *Psophocarpus tetragonolobus* and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

4.3.6 The metal chelating activity of *Sauropus androgynus*

Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus* and standard reference EDTA are shown in Table 4.21. At the concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml, the chelating capacity of EDTA were 94.39 ± 0.26%, 95.65 ± 0.08%, 96.59 ± 0.20%, 98.10 ± 0.08% and 98.63 ± 0.13%, respectively. At the same concentrations, the chelating capacity of crude petroleum ether extracts of *Sauropus androgynus* were 26.49 ± 0.25%, 38.50 ± 0.14%, 48.77 ± 0.37%, 57.93 ± 0.24% and 66.95 ± 0.14%, respectively; crude chloroform extracts exhibited chelating effects of 27.15 ± 0.26%, 44.06 ± 0.58%, 55.65 ± 0.24%, 63.99 ± 0.23% and 70.99 ± 0.14%, respectively, crude methanol extracts exhibited chelating effect of 24.53 ± 0.03%, 28.98 ± 0.39%, 38.23 ± 0.33%, 46.56 ± 0.19% and 50.02 ± 0.36%, respectively and crude water extracts exhibited chelating effect of 4.89 ± 0.33%, 20.78 ± 0.42%, 35.98 ± 0.31%, 39.28 ± 0.30%, 48.14 ± 0.42%, respectively.

As seen, among the four extract, the highest percentage of metal chelating capacity of *Sauropus androgynus* was for the crude chloroform extract following by crude petroleum benzene, methanol and water. They exhibited 70.99%, 66.95%, 50.02% and 48.14% chelation of ferrous ion at 1 mg/ml. Crude methanol extract exhibit more than 50% of metal chelating capacity only at 1 mg/ml. whereas crude methanol extract did not manage to exhibit more than 50% metal chelating capacity even at 1 mg/ml.

Table 4.21: Metal chelating activity of crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus* and standard reference EDTA

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Sweet Leaves/Standard Reference (%)				
	Petroleum Benzene	Chloroform	Methanol	Water	EDTA
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.0625	26.49±0.25	27.15±0.26	24.53±0.03	4.89±0.33	94.39±0.26
0.125	38.50±0.14	44.06±0.58	28.98±0.39	20.78±0.42	95.65±0.08
0.25	48.77±0.37	55.65±0.24	38.23±0.33	35.98±0.31	96.59±0.20
0.5	57.93±0.24	63.99±0.23	46.56±0.19	39.28±0.30	98.10±0.08
1	66.95±0.14	70.99±0.14	50.02±0.36	48.14±0.42	98.63±0.13

Dose-response curves of crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus* with standard reference EDTA are shown in Figure 4.20. As seen, the metal chelating capacities on ferrous ion for all petroleum benzene, chloroform, methanol and water extracts were low at low concentration of 0.0625 mg/ml. Crude petroleum benzene and chloroform extracts showed moderate high metal chelating capacities while crude methanol and water extracts exhibited moderate low metal chelating capacities when compared to that of EDTA at 1 mg/ml.

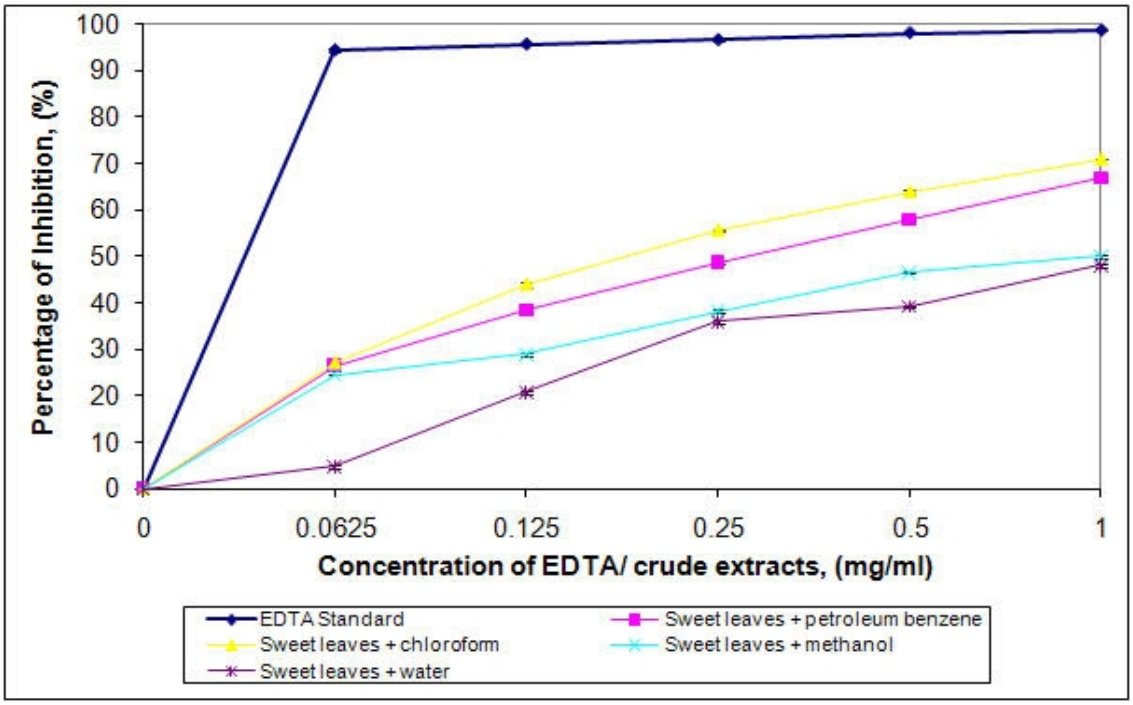


Figure 4.20: Dose-response curves showing chelating activities of *Sauropus androgynus* and ethylenediaminetetraacetic acid (EDTA) as standard reference. Values are expressed as mean ± standard deviation of three independent experiments

Overall, out of the 24 crude extracts tested methanol extract of curry leaves, chloroform extract of sweet potato leaves, petroleum benzene and water extract of celery and petroleum benzene extract of garlic chives showed high metal chelating capacity on ferrous ion and thus are considered as a potential source in preventing the formation of ferrozine-Fe^{2+} complex.

Table 4.22: The chelating capacity on ferrous ion of crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* , at the highest concentration tested, 1 mg/ml in descending order

Chelating capacity on ferrous ion	Percentage of Inhibition of ferrozine-Fe^{2+} complex at 1 mg/ml	Crude Extract of Selected Vegetables
(≥ 80.00%)	**88.60 ± 0.20**	Curry leaves + methanol
	88.56 ± 0.19	Sweet potato leaves + chloroform
	87.46 ± 0.28	Celeries + water
	81.09 ± 0.27	Garlic chives + petroleum benzene
	81.02 ± 0.14	Celeries + petroleum benzene
(50.00-79.99%)	79.78 ± 0.13	Winged beans + chloroform
	79.40 ± 0.14	Sweet potato leaves + petroleum benzene
	78.30 ± 0.23	Curry leaves + chloroform
	78.05± 0.25	Garlic chives +chloroform
	77.02 ± 0.14	Winged beans + petroleum benzene
	73.61 ± 0.22	Curry leaves + petroleum benzene
	73.45 ± 0.17	Celeries + chloroform
	70.99 ± 0.14	Sweet leaves + chloroform
	70.68 ± 0.22	Garlic chives + methanol
	67.02 ± 0.33	Winged beans + water
	66.95 ± 0.14	Sweet leaves + petroleum benzene
	66.81 ± 0.26	Curry leaves + water
	59.52 ± 0.34	Celeries + methanol
	58.94 ± 0.26	Winged beans + methanol
	56.14 ± 0.34	Sweet potato leaves + water
	52.23 ± 0.03	Garlic chives + water
	50.02 ± 0.36	Sweet leaves + methanol
(< 50%)	48.14 ± 0.42	Sweet leaves + water
	46.03 ± 0.30	Sweet potato leaves + methanol

4.4 Haemolysate catalytic assay

In haemolysate catalytic assay, the ability of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* to reduce hydrogen peroxide (H_2O_2) in synergism with haemolysate catalase was assessed. The absorbance at the wavelength of 240 nm was taken as the measure of the amount of H_2O_2 in this assay. The reducing activities of the plant extracts in synergism with haemolysate catalase were determined by measuring the decrease in H_2O_2 absorbance at 240 nm. In this assay, the reducing activities of crude petroleum benzene, chloroform, methanol and water extracts of the 6 selected local vegetables stated above were evaluated at varying concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml.

4.4.1 Determination of the concentration of haemoglobin and total weight of haem in the haemolysate

The concentrations of haemoglobin and total weights of haem in the haemolysate stock used in this haemolysate catalytic assay are shown in Table 4.23. The concentrations of haemoglobin in the haemolysate stocks were calculated according to the Beer-Lambert's law by using an extinction coefficient of 14.6 $M^{-1}cm^{-1}$ for haemoglobin at 577 nm. The absorbance of the haemoglobin in haemolysate stock at 577 nm was 0.0623, giving a haemoglobin concentration of 4.27 mM.

The weights of haem in the haemolysate stocks were determined from the concentrations of haemoglobin obtained by using the molecular weight of 616.49 amu for haem. As stated in Table 4.23, the total weight of haem in haemolysate stock is 7.90 mg.

Table 4.23: The concentration of haemoglobin and weight of haem in haemolysate stock

Haemolysate	Absorbance at 577nm	Concentration of haemoglobin (mM)	Total weight of haem(mg)
Stock	0.0623	4.27	7.90

4.4.2 H_2O_2 – reducing activity of haemolysate catalase as positive reference standard

The activity of the haemolysate catalase alone in reducing H_2O_2 was used as the positive reference standard in the haemolysate catalytic assay. The synergistic effects of the plant extracts and the haemolysate catalase in reducing the activity of H_2O_2 were determined by measuring the difference in reducing activity between positive reference standard and tested extracts. The activity of the positive reference standard, haemolysate catalase alone in the reduction of H_2O_2 was calculated each time before measuring the synergistic effect of the plant extract.

Sodium azide, the inhibitor of catalase was used to confirm the reducing activity of catalase on H_2O_2. The enzyme kinetics of H_2O_2 reduction by unblocked and azide-blocked catalase in different haemolysate stocks are shown in Table 4.24 and illustrated in Figure 4.21. In the presence of functional catalase in haemolysate 1 and 2, the absorbance of H_2O_2 decreased with time as the H_2O_2 was being reduced by haemolysate catalase. When the catalase was blocked by sodium azide, the absorbance of H_2O_2 only decreased slightly with time, showing poor reducing activity on H_2O_2 by catalase in the system. Table 4.24 shows the enzyme kinetics of hydrogen peroxide reduction by unblocked and azide-blocked catalase.

Table 4.24: Enzyme kinetics of hydrogen peroxide reduction by unblocked and azide-blocked catalase in different haemolysate stocks. Values are expressed as mean of three independent experiments

Time (minute)	Absorbance of hydrogen peroxide at 240 nm	
	An Example of Unblocked Catalase used as Standard Reference	Azide-blocked catalase
0	1.304	1.522
1.5	0.355	1.509

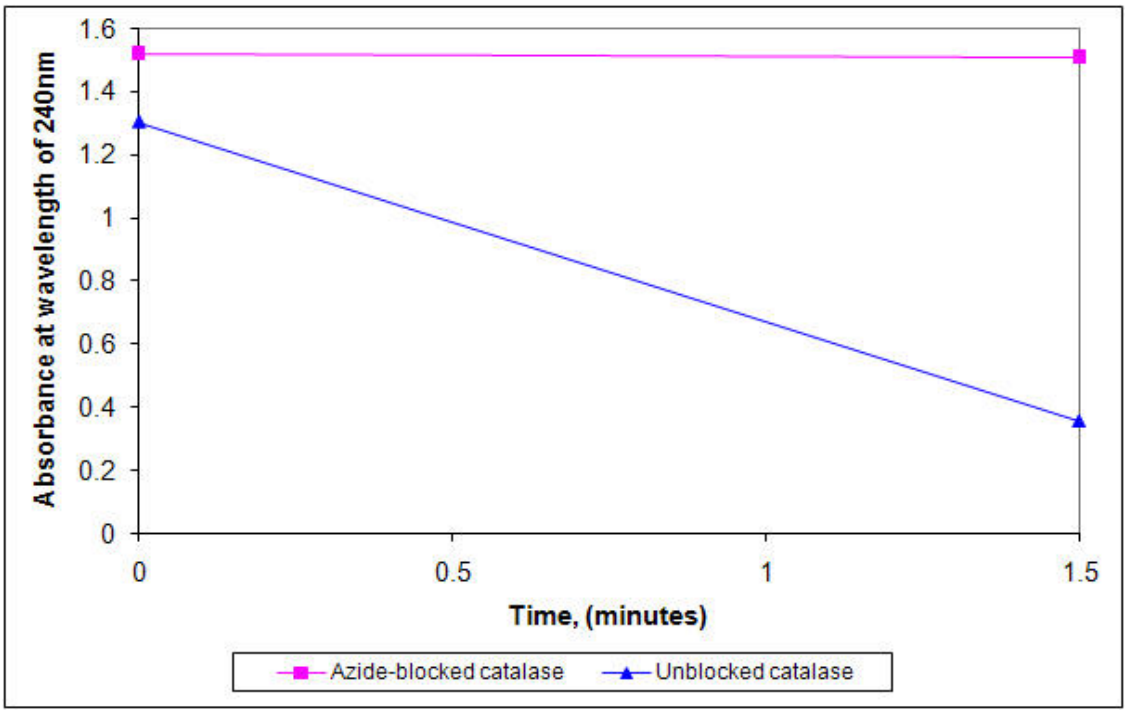


Figure 4.21: Enzyme kinetics of hydrogen peroxide reduction by unblocked and azide-blocked catalasein different haemolysate stocks

4.4.3 H_2O_2 reducing activity of *Allium tuberosum* in synergism with haemolysate catalase

The H_2O_2 reducing activities of crude petroleum benzene, chloroform, methanol, and water extracts of *Allium tuberosum* at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are presented in Table 4.25 and illustrated in Figure 4.22. According to Table 4.25, the percentage of H_2O_2 - reduction for crude petroleum benzene and chloroform extracts ranged from 39.44 ± 1.02% per 50 µg/ml/ 7.90 mg haem , 50.64 ± 2.68% per 100 µg/ml/ 7.90 mg haem , 62.79 ± 1.01 % per 200 µg/ml/ 7.90 mg haem and from 30.11 ± 1.20% per 50 µg/ml/ 7.90 mg haem,

54.85 ± 1.19% per 100 µg/ml/ 7.90 mg haem, 63.45 ± 2.57% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of H_2O_2 - reduction ranged from 39.62 ± 2.84% per 50 µg/ml/ 7.90 mg haem, 49.09 ± 0.84% per 100 µg/ml/ 7.90 mg haem, 63.14 ± 2.60% per 200 µg/ml/ 7.90 mg haem and from 23.49 ± 0.61% per 50 µg/ml/ 7.90 mg haem, 33.60 ± 2.33% per 100 µg/ml/ 7.90 mg haem, 70.19 ± 2.12% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that crude extracts of *A. tuberosum* were able to enhance the activity of haemolysate catalase in decomposing the H_2O_2 into water and oxygen.

Table 4.25: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *A. tuberosum* in synergism with haemolysate catalase

Concentration of crude extract (µg/ml)	Percentage of inhibition (POI) for Garlic Chives (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	39.44 ± 1.02	30.11 ± 1.20	39.62 ± 2.84	23.49 ± 0.61
100	50.64 ± 2.68	54.85 ± 1.19	49.09 ± 0.84	33.60 ± 2.33
200	62.79 ± 1.01	63.45 ± 2.57	63.14 ± 2.60	70.19 ± 2.12

In accordance to Figure 4.22, the percentage of H_2O_2 - reduction in synergism with haemolysate catalase increased with increasing concentrations of *A. tuberosum* extracts. At the highest concentration tested, that is 200 µg/ml, crude water extract exhibited the highest H_2O_2 reducing activity among all the extracts tested, with the percentage of H_2O_2 - reduction of 70.19 ± 2.12% per 200 µg/ml/ 7.90 mg haem. This was followed by crude chloroform, methanol and water extracts which showed similar percentage of H_2O_2 reduction of 63.45 ± 2.57%, 63.14 ± 2.60% and 62.79 ± 1.01% per 200 µg/ml/ 7.90 mg haem.

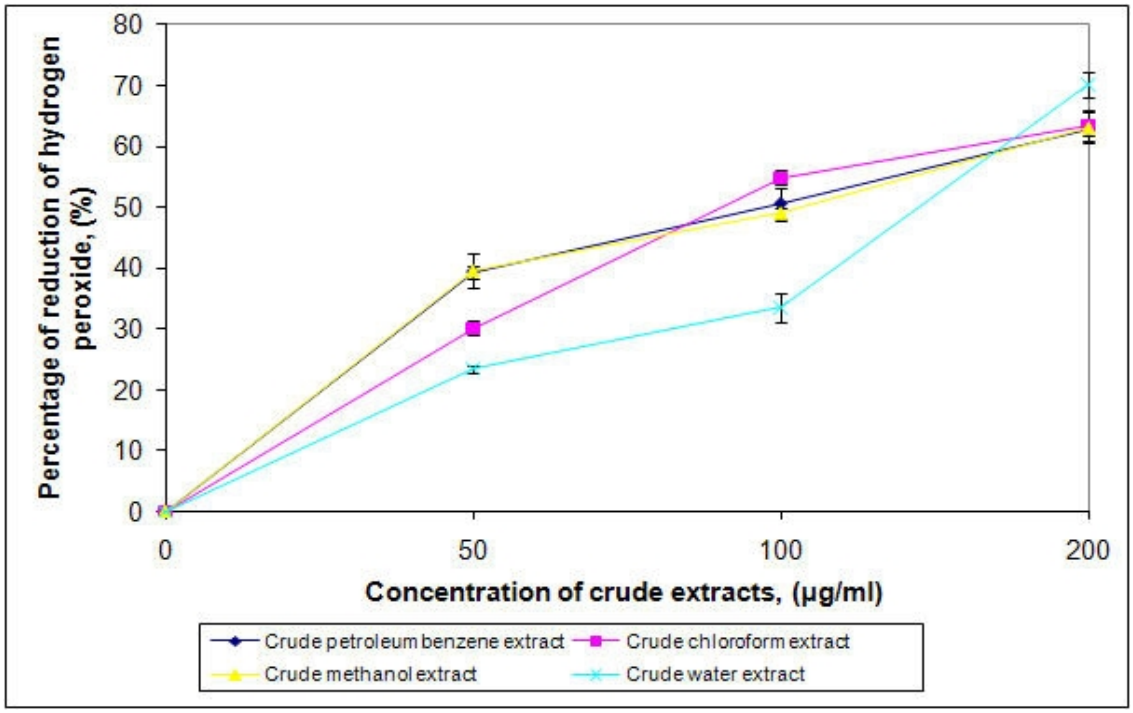


Figure 4.22: Dose-response curves of hydrogen peroxide- reducing activities of *Allium tuberosum* in synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

4.4.4 H_2O_2 reducing activity of *Apium graveolens* (L.) in synergism with haemolysate catalase

The H_2O_2 - reducing activities of crude petroleum benzene, chloroform, methanol, and water extracts of *Apium graveolens* (L.) at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are shown in Table 4.26 and illustrated in Figure 4.23.

For crude petroleum benzene and chloroform extract, the percentage of H_2O_2 - reduction ranged from 16.14 ± 0.72% per 50 µg/ml/ 7.90 mg haem, 20.14 ± 2.15% per 100 µg/ml/ 7.90 mg haem, 43.68 ± 0.92% per 200 µg/ml/ 7.90 mg haem and from 40.77 ± 2.20% per 50 µg/ml/ 7.90 mg haem, 60.69 ± 1.70% per 100 µg/ml/ 7.90 mg haem, 71.86 ± 1.24% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of H_2O_2 - reduction ranged from 30.72 ± 1.34% per 50 µg/ml/ 7.90 mg haem, 35.51 ± 2.28% per 100 µg/ml/ 7.90 mg haem,

64.76 ± 0.90% per 200 µg/ml/ 7.90 mg haem and from 29.74 ± 1.63% per 50 µg/ml/ 7.90 mg haem, 36.93 ± 1.37% per 100 µg/ml/ 7.90 mg haem, 76.49 ± 1.56% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that all the crude extracts of *Apium graveolens* (L.) were able to enhance the catalase activity in reducing H_2O_2.

Table 4.26: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *Apium graveolens* (L.) in synergism with haemolysate catalase

Concentration of crude extract (µg/ml)	Percentage of inhibition (POI) for Celery (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	16.14 ± 0.72	40.77 ± 2.20	30.72 ± 1.34	29.74 ± 1.63
100	20.14 ± 2.15	60.69 ± 1.70	35.51 ± 2.28	36.93 ± 1.37
200	43.68 ± 0.92	71.86 ± 1.24	64.76 ± 0.90	76.49 ± 1.56

Figure 4.23 ilustrates that the H_2O_2 - reduction in synergism with haemolysate catalase increased with increasing concentrations of *Apium graveolens* (L.) extracts. Among all the extracts tested, crude water extract possessed the best synergistic effect with haemolysate catalase in reducing H_2O_2 at 200 µg/ml followed by chloroform, methanol and water. However, crude chloroform of *Apium graveolens* (L.) extracts showed higher percentage of H_2O_2 - reduction at 50 µg/ml and 100 µg/ml compared with water extracts.

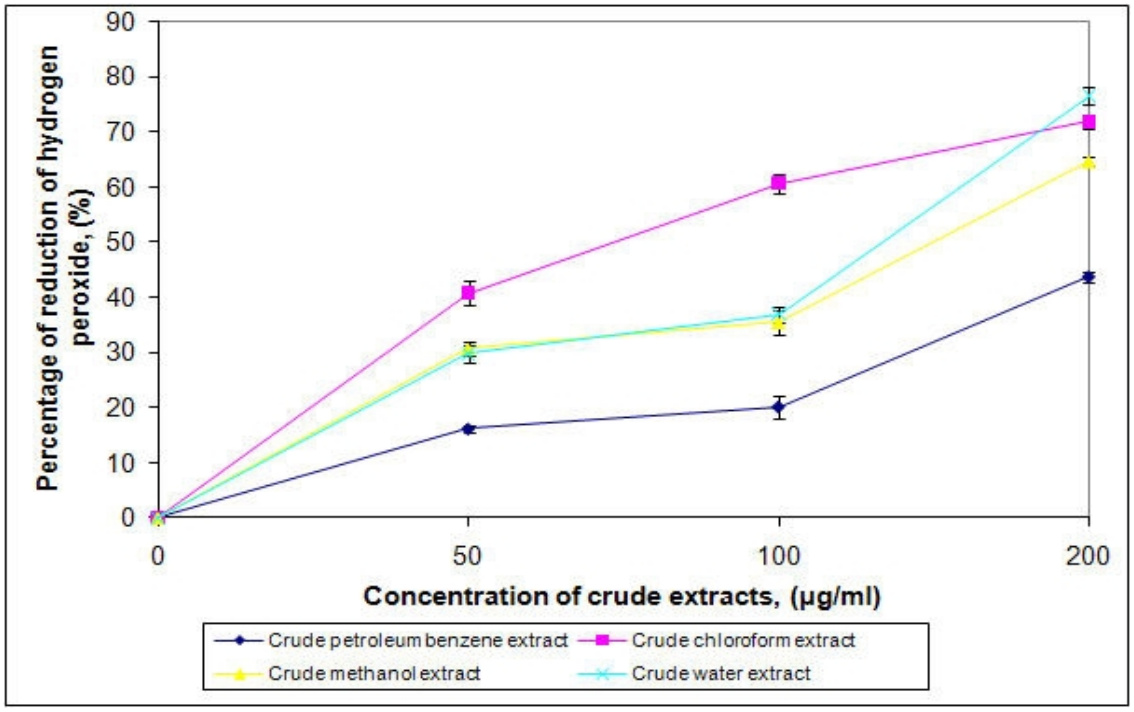


Figure 4.23: Dose-response curves of hydrogen peroxide - reducing activities of *Apium graveolens* (L.) in synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

4.4.5 H_2O_2 - reducing activity of *Ipomoea batatas* (L.) in synergism with haemolysate catalase

The H_2O_2 - reducing activities by crude petroleum benzene, chloroform, methanol, and water extracts of *Ipomoea batatas* (L.) at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are presented in Table 4.27 and illustrated in Figure 4.24. According to the Table 4.27, the percentage of H_2O_2 - reduction for crude petroleum benzene and chloroform extracts ranged from 42.44 ± 1.21% per 50 g/ml/ 7.90 mg haem, 62.24 ± 2.14% per 100 µg/ml/ 7.90 mg haem, 81.15 ± 1.37% per 200 µg/ml/ 7.90 mg haem and from 83.96 ± 1.64% per 50 µg/ml/ 7.90 mg haem, 86.28 ± 2.28% per 100 µg/ml/ 7.90 mg haem, 90.79 ± 0.71% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of H_2O_2 - reduction ranged from 47.26 ± 1.06% per 50 µg/ml/ 7.90 mg haem, 75.77 ± 1.54% per 100 µg/ml/ 7.90 mg haem, 94.03 ± 0.18% per 200 µg/ml/ 7.90 mg haem and from 57.89 ± 0.76% per 50 µg/ml/ 7.90 mg haem, 68.35 ± 2.68% per 100

µg/ml/ 7.90 mg haem, 85.22 ± 0.72% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that all the crude extracts of *A. tuberosum* were able to enhance the activity of haemolysate catalase in decomposing the H_2O_2 into water and oxygen.

Table 4.27: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *Ipomoea batatas* (L.) in synergism with haemolysate catalase

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Sweet Potato Leaves (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	42.44 ± 1.21	83.96 ± 1.64	47.26 ± 1.06	57.89 ± 0.76
100	66.24 ± 2.14	86.28 ± 0.19	75.77 ± 1.54	68.35 ± 2.68
200	81.15 ± 1.37	90.79 ± 0.71	94.03 ± 0.18	85.22 ± 0.72

As illustrated in Figure 4.24, the percentage of H_2O_2 - reduction in synergism with haemolysate catalase increased with increasing concentrations of *Ipomoea batatas* (L.) extracts. At the highest concentration tested, that is 200 µg/ml, crude methanol extract exhibited the highest H_2O_2 reducing activity among all the extracts tested, with the percentage of reduction of H_2O_2 reaching 94.03 ± 0.18% per 200 µg/ml/ 7.90 mg haem. This was followed by crude chloroform, water and petroleum benzene extracts which all show very good percentage of H_2O_2 - reduction of 90.79 ± 0.71%, 85.22 ± 0.72% and 81.15 ± 1.37% per 200 µg/ml/ 7.90 mg haem. All crude extracts of sweet potato leaves has high potential in enhancing the activity of haemolysate catalase in decomposing the H_2O_2 into water and oxygen as the percentage of H_2O_2 reduction.

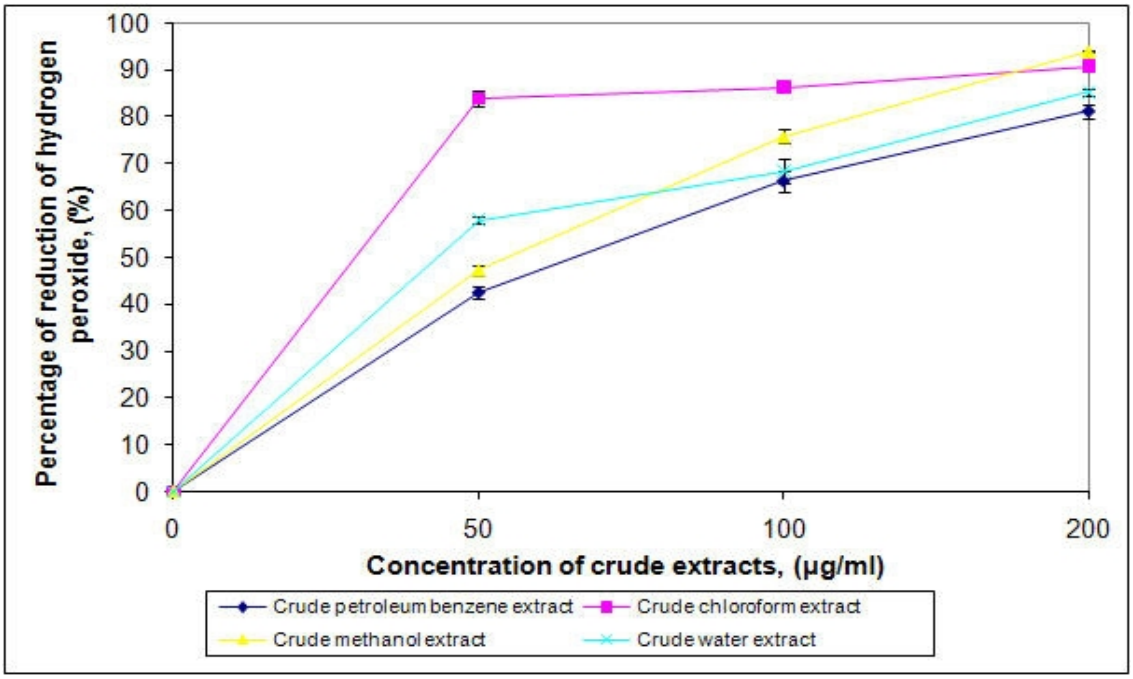


Figure 4.24: Dose-response curves of hydrogen peroxide- reducing activities of *Ipomoea batatas* (L.) in synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

4.4.6 H_2O_2 reducing activity of *Murraya koenigii* (L.) in synergism with haemolysate catalase

The H_2O_2 - reducing activities of crude petroleum benzene, chloroform, methanol, and water extracts of *Murraya koenigii* (L.) at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are shown in Table 4.28 and illustrated in Figure 4.25.

For crude petroleum benzene and chloroform extract, the percentage of H_2O_2 - reduction ranged from 49.03 ± 1.68% per 50 µg/ml/ 7.90 mg haem, 66.68 ± 1.45% per 100 µg/ml/ 7.90 mg haem, 76.39 ± 1.01% per 200 µg/ml/ 7.90 mg haem and from 38.44 ± 0.39% per 50 µg/ml/ 7.90 mg haem, 70.34 ± 0.70% per 100 µg/ml/ 7.90 mg haem, 87.36 ± 0.77% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of H_2O_2 - reduction ranged from 12.60 ± 1.06% per 50 µg/ml/ 7.90 mg haem, 37.60 ± 1.19% per 100 µg/ml/ 7.90 mg haem,

74.65 ± 1.19% per 200 µg/ml/ 7.90 mg haem and from 17.77 ± 1.67% per 50 µg/ml/ 7.90 mg haem, 38.72± 1.63% per 100 µg/ml/ 7.90 mg haem, 74.45 ± 2.32% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that all of the crude extracts of curry leaves were able to enhance the catalase activity in reducing H_2O_2.

Table 4.28: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *Murraya koenigii* (L.) in synergism with haemolysate catalase

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Curry Leaves (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	49.03 ± 1.68	38.44 ± 0.39	12.60 ± 1.06	17.77 ± 1.67
100	66.68 ± 1.45	70.34 ± 0.70	37.60 ± 1.19	38.72 ± 1.63
200	76.39 ± 1.01	87.36 ± 0.77	74.65 ± 1.19	74.45 ± 2.32

Figure 4.25 illustrates that the H_2O_2 – reducing activities in synergism with haemolysate catalase increased with increasing concentration of *Murraya koenigii* (L.) extracts. Among all the extracts tested, crude chloroform extract possessed the best synergistic effect with haemolysate catalase in reducing H_2O_2 at 200 µg/ml followed by petroleum benzene, methanol and water which showed similar percentage of H_2O_2 reducing activities of of 76.39 ± 1.01%, 74.65 ± 1.19%, and 74.45 ± 2.32%, per 200 µg/ml/ 7.90 mg haem. However, crude petroleum benzene of curry leaves extracts showed higher percentage of H_2O_2 - reduction at 50 µg/ml compared with other extracts of curry leaves.

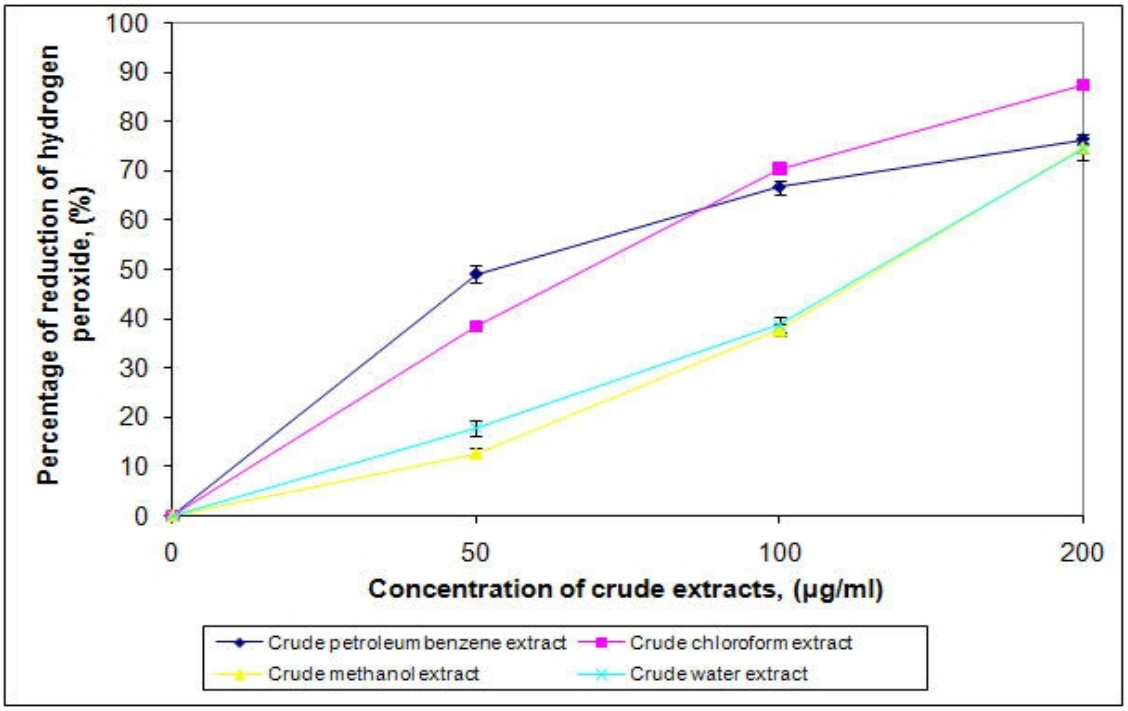


Figure 4.25: Dose-response curves of hydrogen peroxide- reducing activities of *Murraya koenigii* (L.) in synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

4.4.7 H_2O_2 reducing activity of *Psophocarpus tetragonolobus* in synergism with haemolysate catalase

The H_2O_2 - reducing activities by crude petroleum benzene, chloroform, methanol, and water extracts of *Psophocarpus tetragonolobus* at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are presented in Table 4.29 and illustrated in Figure 4.26. According to Table 4.29, the percentage of H_2O_2 – reduction for crude petroleum benzene and chloroform extracts ranged from 37.91 ± 1.35% per 50 µg/ml/ 7.90 mg haem, 67.45 ± 0.37% per 100 µg/ml/ 7.90 mg haem, 77.71 ± 0.19% per 200 µg/ml/ 7.90 mg haem and from 72.72 ± 2.58% per 50 µg/ml/ 7.90 mg haem, 80.64 ± 1.14% per 100 µg/ml/ 7.90 mg haem, 92.53 ± 0.99% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of reduction of H_2O_2 ranged from 40.98 ± 1.14% per 50 µg/ml/ 7.90 mg haem, 51.27 ± 0.21% per 100 µg/ml/ 7.90 mg haem, 85.34 ± 0.39% per 200 µg/ml/ 7.90 mg haem and from 68.42 ± 1.42% per 50 µg/ml/ 7.90 mg haem, 79.69

± 1.05% per 100 µg/ml/ 7.90 mg haem, 98.00 ± 0.16% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that all the crude extracts of winged beans were able to enhance the activity of haemolysate catalase in decomposing the H_2O_2 into water and oxygen.

Table 4.29: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *Psophocarpus tetragonolobus* in synergism with haemolysate catalase

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Winged Bean (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	37.91 ± 1.35	72.72 ± 2.58	40.98 ± 1.14	68.42 ± 1.42
100	67.45 ± 0.37	80.64 ± 1.14	51.27 ± 0.21	79.69 ± 1.05
200	77.71 ± 0.19	92.53 ± 0.99	85.34 ± 0.39	98.00 ± 0.16

In accordance to Figure 4.26, the percentage of H_2O_2 - reduction in synergism with haemolysate catalase increased with increasing concentrations of *P. tetragonolobus* extracts. At the highest concentration tested, that is 200 µg/ml, crude water extract exhibited the highest reducing activity on H_2O_2 among all the extracts tested, with the percentage of reduction of H_2O_2 of 98.00 ± 0.16% per 200 µg/ml/ 7.90 mg haem. This was followed by crude chloroform; methanol and petroleum benzene extracts which enhanced reduction of H_2O_2 by 92.53 ± 0.99%, 85.34 ± 0.39%, and 77.71 ± 0.19% per 200 µg/ml/ 7.90 mg haem.

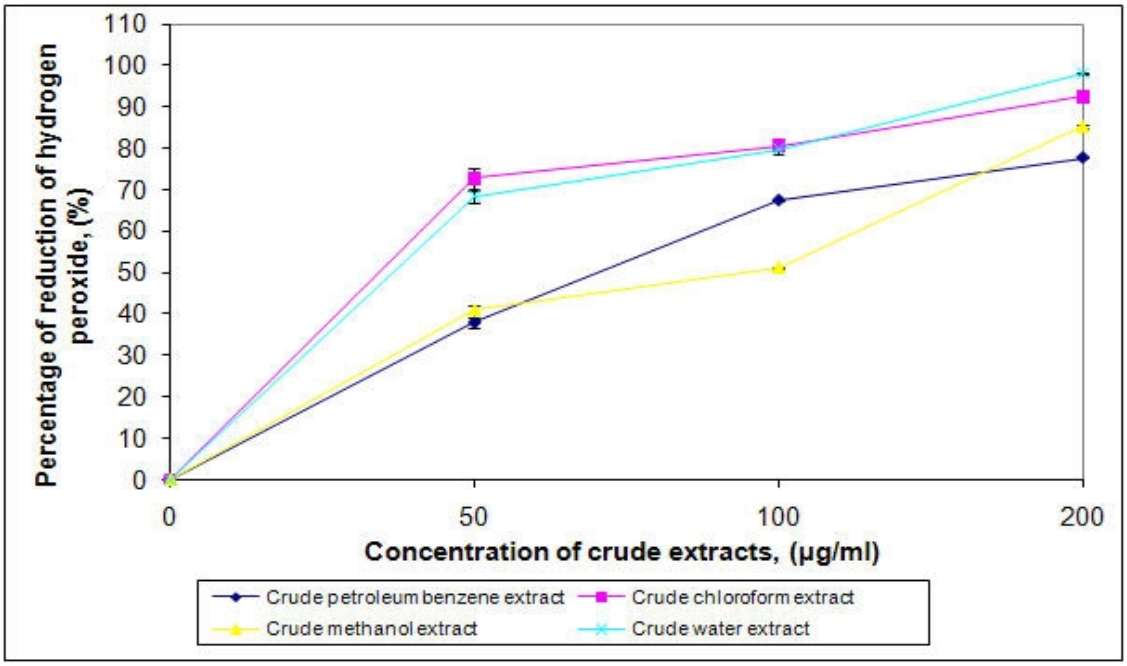


Figure 4.26: Dose-response curves of hydrogen peroxide- reducing activities of *Psophocarpus tetragonolobus* in synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

4.4.8 H_2O_2 reducing activity of *Sauropus androgynus* in synergism with haemolysate catalase

The H_2O_2 - reducing activities by crude petroleum benzene, chloroform, methanol, and water extracts of *Sauropus androgynus* at varying concentrations (50 µg/ml, 100 µg/ml and 200 µg/ml) in synergism with haemolysate catalase are shown in Table 4.30 and illustrated in Figure 4.27.

For crude petroleum benzene and chloroform extract, the percentage of H_2O_2 reduction ranged from 13.03 ± 2.53% per 50 µg/ml/ 7.90 mg haem, 52.98 ± 1.25% per 100 µg/ml/ 7.90 mg haem, 62.40 ± 1.00% per 200 µg/ml/ 7.90 mg haem and from 61.35 ± 0.96% per 50 µg/ml/ 7.90 mg haem, 62.15 ± 0.27% per 100 µg/ml/ 7.90 mg haem, 76.29 ± 0.91% per 200 µg/ml/ 7.90 mg haem, respectively. On the other hand, for crude methanol and water extract, the percentage of H_2O_2 - reduction ranged from 12.51 ± 2.56% per 50 µg/ml/ 7.90 mg haem, 53.60 ± 2.23% per 100 µg/ml/ 7.90 mg haem, 78.30 ± 0.96% per 200 µg/ml/ 7.90 mg haem and from 30.25 ± 1.95% per 50 µg/ml/

7.90 mg haem, 58.77 ± 1.48% per 100 µg/ml/ 7.90 mg haem, 76.52 ± 2.87% per 200 µg/ml/ 7.90 mg haem, respectively. The results revealed that all the crude extracts of sweet leaves were able to enhance the catalase activity in reducing H_2O_2.

Table 4.30: Percentage of hydrogen peroxide reduction by crude petroleum benzene, chloroform, methanol and water extracts of *Sauropus androgynus* in synergism with haemolysate catalase

Concentration of crude extract (mg/ml)	Percentage of inhibition (POI) for Sweet Leaves (%)			
	Petroleum Benzene	Chloroform	Methanol	Water
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	13.03 ± 2.53	61.35 ± 0.96	12.51 ± 2.56	30.25 ± 1.95
100	52.98 ± 1.25	62.15 ± 0.27	53.60 ± 2.23	58.77 ± 1.48
200	62.40 ± 1.00	76.29 ± 0.91	78.30 ± 0.96	76.52 ± 2.87

Figure 4.27 illustrates that the percentage of H_2O_2 reduction in synergism with haemolysate catalase increased with increasing concentrations of *Sauropus androgynus* extracts. Among all the extracts tested, crude methanol extract possessed the best synergistic effect with haemolysate catalase in reducing H_2O_2 at 200 µg/ml followed by crude water and chloroform extracts. The H_2O_2 reducing activities were enhanced by 78.30 ± 0.96%, 76.52 ± 2.87%, and 76.29 ± 0.91%, per 200 µg/ml/ 7.90 mg haem. On the other hand, crude petroleum benzene extracts showed slightly lower percentage of H_2O_2 reduction at 200 µg/ml/ 7.90 mg, that is 62.40 ± 1.00%.

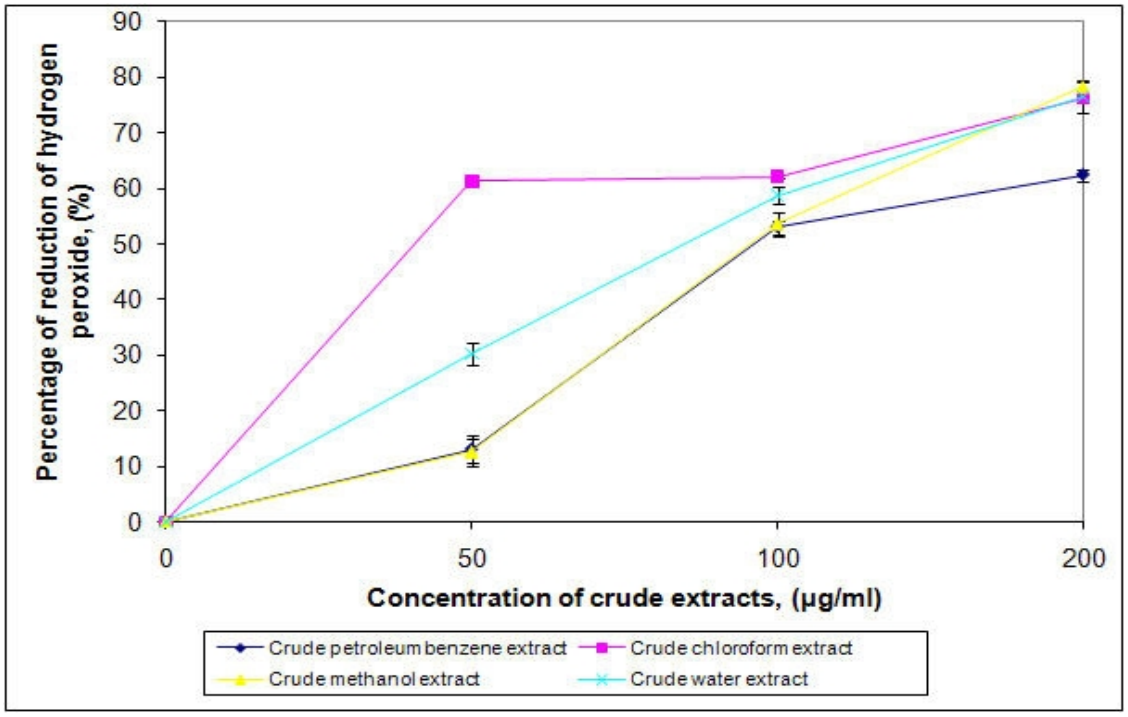


Figure 4.27: Dose-response curves of hydrogen peroxide- reducing activities of *Sauropus androgynus* synergism with haemolysate catalase. Values are expressed as mean ± standard deviation of three independent experiments

Table 4.31 shows the reducing capabilities of hydrogen peroxide by crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* at the highest concentration tested, 200 µg/ml/ 7.90 mg. Overall, out of the 24 crude extracts tested, crude water, chloroform and methanol extracts of winged beans exhibited the best reducing capacities on hydrogen peroxide followed by crude extracts of sweet potato leaves where both these plants crude extracts exhibited good, ie more than 80 percentage of H_2O_2 reduction except for petroleum benzene extract of winged bean which exhibited moderate capabilities of relatively high that is 77.71 ± 0.19% at 200 µg/ml/ 7.90 mg haem. Crude extracts of curry leaves which was seen as a potential antioxidant source in the last three assays, also exhibited high and moderate percentage of reduction of hydrogen peroxide with the values highlighted in Table 4.31.

Table 4.31: The reducing capabilities of hydrogen peroxide by crude petroleum benzene, chloroform, methanol and water extracts of *Allium tuberosum*, *Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus*, and *Sauropus androgynus* at the highest concentration tested, 200 µg/ml/ 7.90 mg haem in descending order

Reducing capacities on hydrogen peroxide	Percentage of Reduction (%) of hydrogen peroxide (H_2O_2)	Crude Extract of Selected Vegetables
(≥ 80.00%)	98.00 ± 0.16	Winged beans + water
	94.03 ± 0.18	Sweet potato leaves + methanol
	92.53 ± 0.99	Winged beans + chloroform
	90.79 ± 0.71	Sweet potato leaves + chloroform
	87.36 ± 0.77	Curry leaves + chloroform
	85.34 ± 0.39	Winged beans + methanol
	85.22 ± 0.72	Sweet potato leaves + water
	81.15 ± 1.37	Sweet potato leaves + petroleum benzene
(50.00-79.99%)	78.30 ± 0.96	Sweet leaves + methanol
	77.71 ± 0.19	Winged beans + petroleum benzene
	76.52 ± 2.87	Sweet leaves + water
	76.49 ± 1.56	Celeries + water
	76.39 ± 1.01	Curry leaves + petroleum benzene
	76.29 ± 0.91	Sweet leaves + chloroform
	74.65 ± 1.19	Curry leaves + methanol
	74.45 ± 2.32	Curry leaves + water
	71.86 ± 1.24	Celeries + chloroform
	70.19 ± 2.12	Garlic chives + water
	64.76 ± 0.90	Celeries + methanol
	63.45 ± 2.57	Garlic chives +chloroform
	63.14 ± 2.60	Garlic chives + methanol
	62.79 ± 1.01	Garlic chives + petroleum benzene
	62.40 ± 1.00	Sweet leaves + petroleum benzene
(< 50%)	43.68 ± 0.92	Celeries + petroleum benzene

4.4.9 Comparison of H_2O_2 - reducing activities of crude extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* in synergism with haemolysate catalase at varying concentrations

The reducing activities on H_2O_2 by crude extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* in synergism with haemolysate catalase at 50 µg/ml are presented and compared in Figure 4.28. At the lowest concentration tested of 50 µg/ml, the crude methanol extract of *Murraya koenigii* (L.) and *Sauropus androgynus* exhibited relatively lower H_2O_2 reducing activities in comparison to other extracts. On the other hand, the crude chloroform of *Ipomoea batatas* (L.) showed very effective H_2O_2 reducing activity even at low concentration, with the percentage of H_2O_2 reduction reaching up to 83.96 ± 1.64% per 50 µg/ml/ 7.90 mg haem at 50 µg/ml.

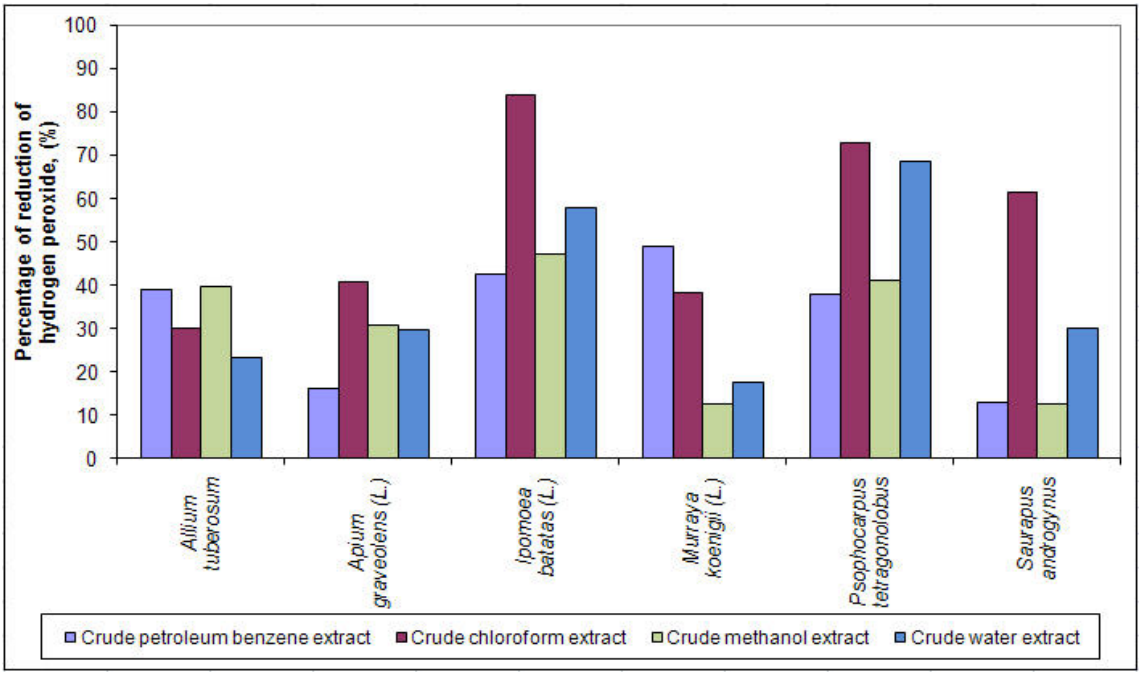


Figure 4.28: H_2O_2 - reducing activities of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* synergism with haemolysate catalase at 50 µg/ml

Figure 4.29 illustrates and compares the H_2O_2 reducing activities of crude extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus, and Sauropus androgynus* in synergism with haemolysate catalase at the concentration 100 µg/ml. At the concentration of 100 µg/ml, the percentage of H_2O_2 reduction by all these extracts increased with crude chloroform of *Ipomoea batatas* (L.) showing very effective H_2O_2 reducing activity with the percentage of H_2O_2 reduction reaching up to 86.28 ± 0.19% per 100 µg/ml/ 7.90 mg haem. At the same concentration of 100µg/ml, reducing activity on H_2O_2 by methanol extracts of *Murraya koenigii* (L.) was the lowest with the percentage of H_2O_2 reduction of only 37.60 ± 1.19% per 100 µg/ml/ 7.90 mg haem.

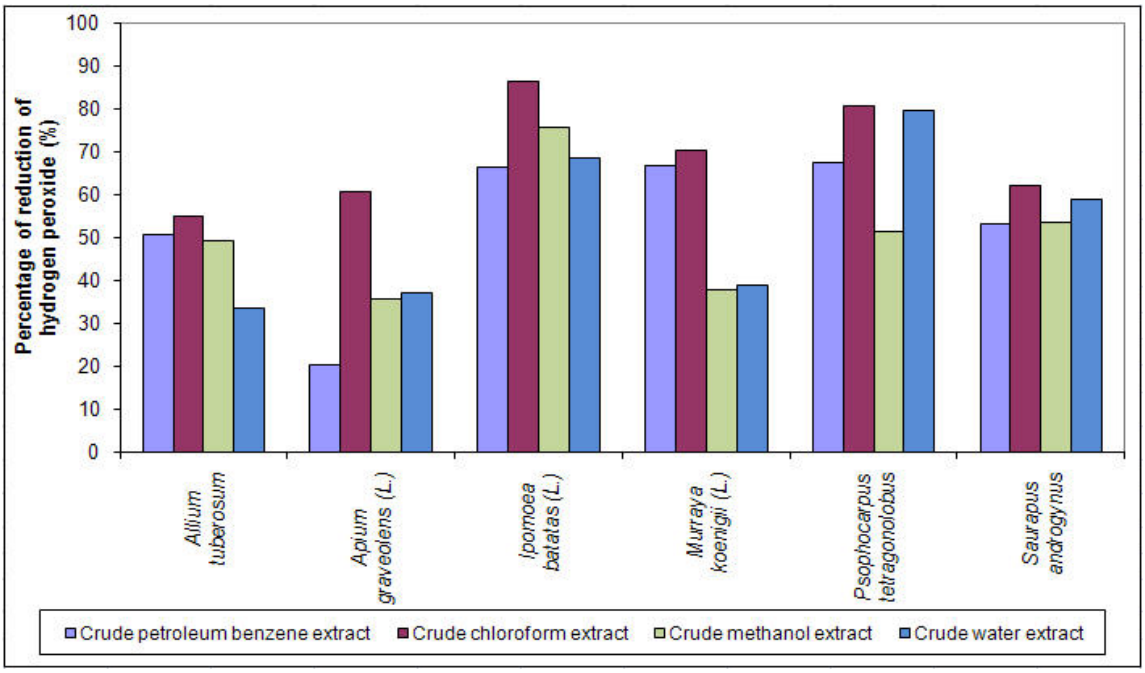


Figure 4.29: H_2O_2 - reducing activities of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* synergism with haemolysate catalase at 100 µg/ml

Figure 4.30 shows and compares the H_2O_2 reducing activities of crude extracts of *Allium tuberosum*, *Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus*, and *Sauropus androgynus* in synergism with haemolysate catalase at the concentration 200 µg/ml. At these high concentration of 200 µg/ml, all the extracts of the selected vegetables exhibited higher reducing activities on H_2O_2 in synergism with haemolysate catalase. Crude water extract of *Psophocarpus tetragonolobus* exhibited very high reducing activities on H_2O_2 with the percentage of H_2O_2 reduction reaching up to 98.00 ± 0.16% per 200 µg/ml/ 7.90 mg haem at 200 µg/ml. On the other hand, crude petroleum benzene of *Apium graveolens* (L.) showed the lowest reducing activity on H_2O_2 with the percentage of H_2O_2 reduction of only 43.68 ± 0.92% per 200 µg/ml/ 7.90 mg haem at 200 µg/ml.

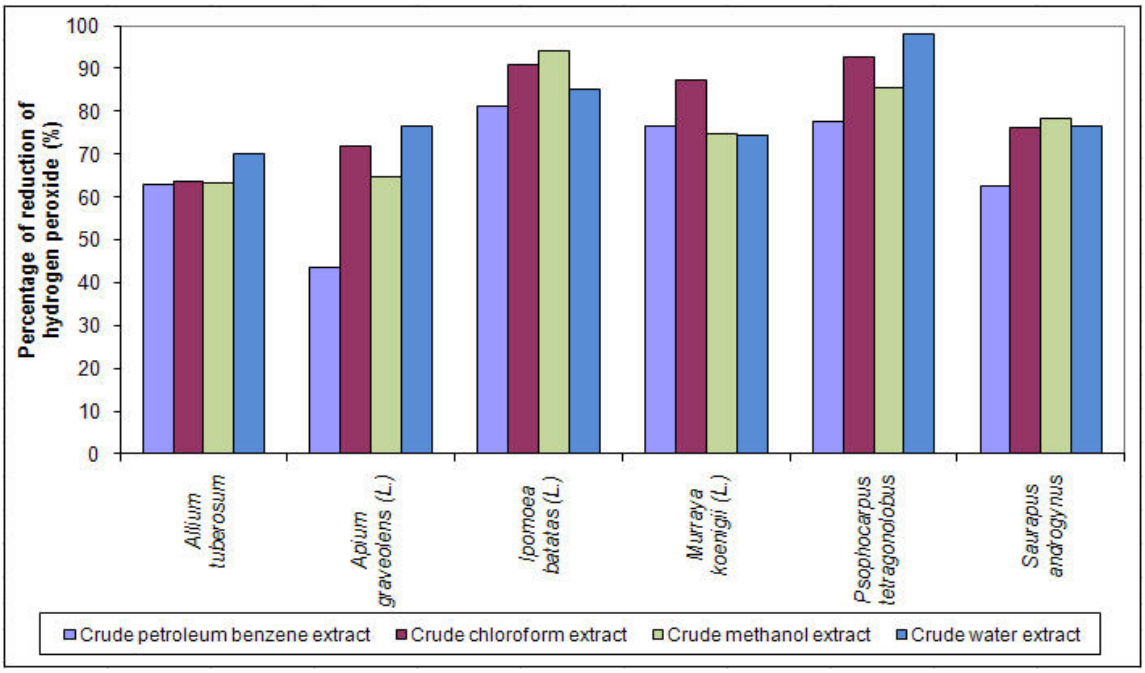


Figure 4.30: H_2O_2 - reducing activities of *Allium tuberosum*, *Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus*, and *Sauropus androgynus* synergism with haemolysate catalase at 200 µg/ml

4.5 Lipid Hydroperoxide assay

In lipid hydroperoxide assay, the level of lipid hydroperoxide of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* in synergism with Lipid Hydroperoxide standard was measured. The absorbance at the wavelength of 500 nm was taken as the measure of the amount of lipid hydroperoxide in this assay. In this assay, the lipid hydroperoxide levels of crude petroleum benzene, chloroform and methanol extracts of the 6 selected local vegetables stated above were evaluated at the concentration of 25 nmol using plate reader provided in the assay kit.

4.5.1 Lipid Hydroperoxide Standard

Table 4.32 showed the hydroperoxide values of the standard tubes and the concentration of the lipid hydroperoxide standard.

The hydroperoxide values were increased with increasing concentrations of lipid hydroperoxides. The hydroperoxide values at 0 nmol, 0.5 nmol, 1 nmol, 1.5 nmol, 2 nmol, 3 nmol, 4 nmol and 5 nmol were 0.113, 0.396, 0.928, 1.394, 2.126, 2.975, 4.223 and 4.839 respectively.

As seen in Table 4.32, the highest hydroperoxide value of the standard was **4.839 nmol** obtained at 5 nmol. The lowest hydroperoxide value of the standard was **0.113 nmol** obtained at 0 nmol.

Figure 4.31 showed that corrected absorbance was used to plot a typical standard curve. The hydroperoxide values of the sample tubes (HPST) was calculated using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample. As seen in Figure 4.31, the standard curve seemed

to increase gradually from the concentration of 0 nmol to 5 nmol. This means that the hydroperoxide values increased with increasing concentration of the lipid hydroperoxide standard.

Table 4.32: Hydroperoxide values of the standard tubes at different concentrations range from 0 to 5 nmol

Concentration of Lipid Hydroperoxide (nmol)	Absorbance at 500 nm				Corrected Absorbance	HPST (nmol)	Concentration of Hydroperoxides (μM)
	Replicate 1	Replicate 2	Replicate 3	Average			
0	0.298	0.287	0.291	0.292 ± 0.006	0	0.113	0.452
0.5	0.275	0.278	0.273	0.275 ± 0.003	0.017	0.396	1.584
1.0	0.238	0.241	0.251	0.243 ± 0.007	0.049	0.928	3.712
1.5	0.210	0.219	0.215	0.215 ± 0.005	0.077	1.394	5.576
2.0	0.171	0.168	0.174	0.171 ± 0.003	0.121	2.126	8.504
3.0	0.120	0.118	0.123	0.120 ± 0.003	0.172	2.975	11.900
4.0	0.045	0.042	0.049	0.045 ± 0.004	0.247	4.223	16.892
5.0	0.005	0.008	0.010	0.008 ± 0.003	0.284	4.839	19.356

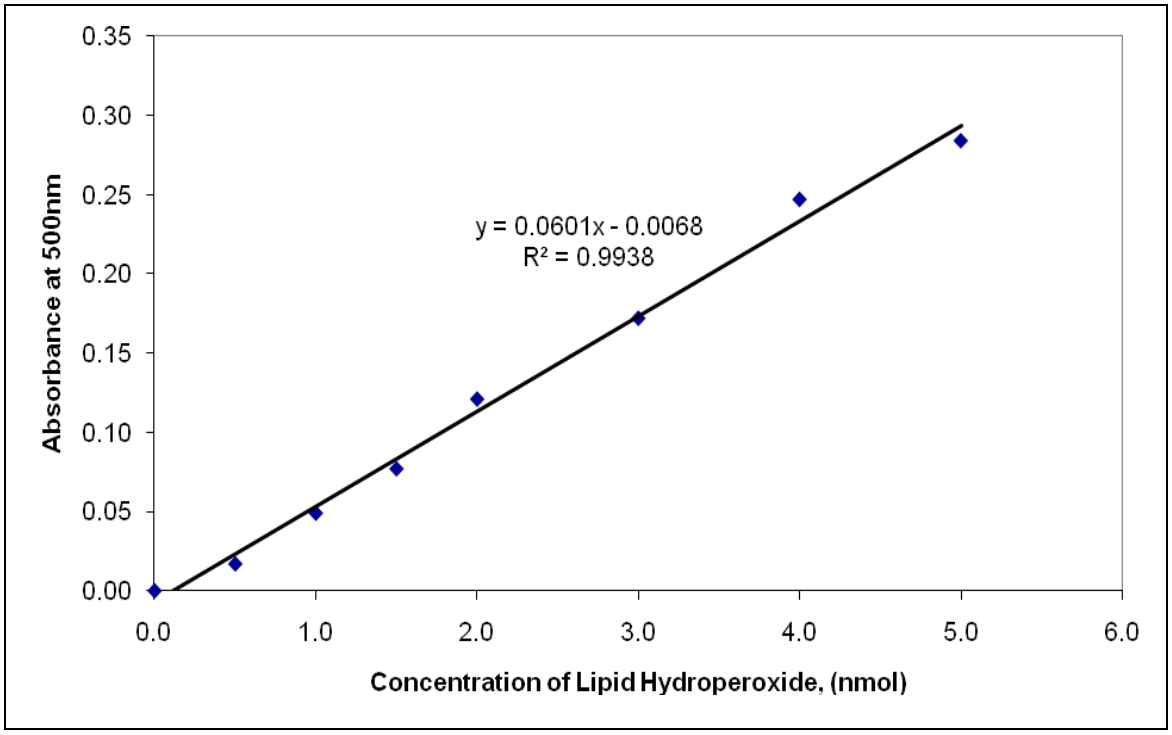


Figure 4.31: The standard curve of lipid hydroperoxide standard in lipid hydroperoxide assay

4.5.2 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Allium tuberosum*

Crude petroleum benzene, chloroform and methanol extracts of *Allium tuberosum* were screened to measure their lipid hydroperoxide level at 25 nmol. The average absorbance,corrected absorbance,lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Allium tuberosum* tested in 3 different crude extracts (petroleum ether, chloroform and methanol) are shown in Table 4.33. Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate these average absorbance.

Table 4.33: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Allium tuberosum*

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (µM)
Petroleum Benzene	0.275	0.271	0.269	0.272 ± 0.003	0.020	0.446	1.784
Chloroform	0.252	0.243	0.249	0.248 ± 0.005	0.044	0.845	3.380
Methanol	0.269	0.256	0.267	0.264 ± 0.007	0.028	0.579	2.316

As shown in Table 4.33, all 3 extracts hydroperoxide level ranges between 0.446 nmol to 0.845 nmol. Out of the 3 extracts of garlic chives, chloroform extract showed highest hydroperoxide value of 0.845 nm whereas petroleum benzene extract of garlic chives showed lowest hydroperoxide value of 0.446 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are very low.

4.5.3 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Apium graveolens* (L.)

The average absorbance, corrected absorbance, lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Apium graveolens* (L.) tested in 3 different crude extracts (petroleum ether, chloroform and methanol) are shown in Table 4.34. Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate these average absorbance.

Table 4.34: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Apium graveolens* (L.)

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (µM)
Petroleum Benzene	0.230	0.220	0.238	0.229 ± 0.009	0.063	1.161	4.644
Chloroform	0.159	0.163	0.150	0.157 ± 0.007	0.135	2.359	9.436
Methanol	0.285	0.280	0.271	0.279 ± 0.007	0.013	0.329	1.316

As shown in Table 4.34, all 3 extracts hydroperoxide level ranges between 0.329 nmol to 2.359 nmol. Out of the 3 crude extracts of celery, chloroform extract showed highest hydroperoxide value of 2.359 nmol whereas methanol extract of celery showed lowest hydroperoxide value of 0.329 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level especially for the chloroform extract of celery.

4.5.4 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Ipomoea batatas*

Table 4.35 showed the average absorbance, corrected absorbance, lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Ipomoea batatas* tested in 3 different crude extracts (petroleum benzene, chloroform and methanol) at 25 nmol.Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate this average absorbance.

Table 4.35: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Ipomoea batatas*

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (μM)
Petroleum Benzene	0.224	0.202	0.199	0.208 ± 0.014	0.084	1.511	6.044
Chloroform	0.120	0.128	0.109	0.119 ± 0.010	0.173	2.992	11.968
Methanol	0.107	0.112	0.123	0.114 ± 0.008	0.178	3.075	12.300

As shown in Table 4.3.5, all 3 extracts of sweet potato leaves hydroperoxide level ranges between 1.511 nmol to 3.075 nmol. Out of the 3 crude extracts of sweet potato leaves, methanol extract showed highest hydroperoxide value of 3.075 nmol whereas petroleum benzene extract showed lowest hydroperoxide value of 1.511 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level especially for the chloroform and methanol extracts of sweet potato leaves.

4.5.5 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Murraya koenigii*

Table 4.36 showed the average absorbance, corrected absorbance, lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Murraya koenigii* tested in 3 different crude extracts (petroleum benzene, chloroform and methanol) at 25 nmol. Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate this average absorbance.

Table 4.36: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Murraya koenigii*

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (µM)
Petroleum Benzene	0.191	0.182	0.179	0.184 ± 0.006	0.108	1.910	7.640
Chloroform	0.192	0.185	0.180	0.186 ± 0.006	0.106	1.877	7.508
Methanol	0.137	0.128	0.130	0.132 ± 0.005	0.160	2.775	11.100

As shown in Table 4.36, all 3 extracts of curry leaves hydroperoxide level ranges between 1.877 nmol to 2.775 nmol. Out of the 3 crude extracts of curry leaves, methanol extract showed highest hydroperoxide value of 2.775 nmol whereas chloroform extract showed lowest hydroperoxide value of 1.877 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level especially for the methanol extracts of curry leaves.

4.5.6 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Psophocarpus tetragonolobus*

The average absorbance, corrected absorbance, lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Psophocarpus tetragonolobus* tested in 3 different crude extracts (petroleum benzene, chloroform and methanol) are shown in Table 4.37. Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate these average absorbance.

Table 4.37: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Psophocarpus tetragonolobus*

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (µM)
Petroleum Benzene	0.154	0.151	0.147	0.151 ± 0.004	0.141	2.459	9.836
Chloroform	0.189	0.181	0.179	0.183 ± 0.005	0.109	1.927	7.708
Methanol	0.205	0.212	0.215	0.211 ± 0.005	0.081	1.461	5.844

As shown in Table 4.37, all 3 extracts of winged beans hydroperoxide level ranges between 1.461 nmol to 2.459 nmol. Out of the 3 crude extracts of winged beans, petroleum benzene extract showed highest hydroperoxide value of 2.459 nmol whereas methanol extract showed lowest hydroperoxide value of 1.461 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level especially for the petroleum benzene extracts of winged beans.

4.5.7 Measurement of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Sauropus androgynus*

The average absorbance, corrected absorbance, lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of *Sauropus androgynus* tested in 3 different crude extracts (petroleum benzene, chloroform and methanol) are shown in Table 4.38. Mean values of the absorbance reading or optical density at 500 nm from three independent test runs were used to calculate these average absorbance.

Table 4.38: Hydroperoxide values of crude petroleum benzene, chloroform and methanol extracts of *Sauropus androgynus*

Type of Extract	Replicate 1	Replicate 2	Replicate 3	Average	Corrected absorbance	HPST (nmol)	Concentration of Hydroperoxides (µM)
Petroleum Benzene	0.153	0.149	0.161	0.154 ± 0.006	0.138	2.409	9.636
Chloroform	0.192	0.182	0.188	0.187 ± 0.005	0.105	1.860	7.440
Methanol	0.202	0.199	0.210	0.204 ± 0.006	0.088	1.577	6.308

As shown in Table 4.38, all 3 extracts of sweet leaves hydroperoxide level ranges between 1.577 nmol to 2.409 nmol. Out of the 3 crude extracts of sweet leaves, petroleum benzene extract showed highest hydroperoxide value of 2.409 nmol whereas methanol extract showed lowest hydroperoxide value of 1.577 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level especially for the petroleum benzene extracts of sweet leaves.

4.5.8 Comparison of Lipid Hydroperoxide values of petroleum benzene, chloroform and methanol extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus* and *Sauropus androgynus*

Crude petroleum ether, chloroform, methanol and water extracts of *Allium tuberosum, Apium graveolens* (L.), *Ipomoea batatas* (L.), *Murraya koenigii* (L.), *Psophocarpus tetragonolobus,* and *Sauropus androgynus* were measured for their hydroperoxide levels at 25 nmol. The lipid hyroperoxide values for the samples tube and concentration of hydroperoxides of all the six selected local vegetables tested in 3 different crude extracts (petroleum ether, chloroform and methanol) are shown in Table 4.39.

Table 4.39: The hydroperoxide values of all 18 crude extracts at 25 nmol

Type Of Extract	HPST (nmol)	Concentration of hydroperoxides (µM)
1) *Allium tuberosum*		
• Petroleum Benzene	0.446	1.784
• Chloroform	0.845	3.380
• Methanol	0.579	2.316
2) *Apium graveolens* (L.)		
• Petroleum Benzene	1.161	4.644
• Chloroform	2.359	9.436
• Methanol	0.329	1.316
3) *Ipomoea batatas* (L.)		
• Petroleum Benzene	1.511	6.044
• Chloroform	2.992	11.968
• Methanol	3.075	12.300
4) *Murraya koenigii* (L.)		
• Petroleum Benzene	1.910	7.640
• Chloroform	1.877	7.508
• Methanol	2.775	11.100
5) *Psophocarpus tetragonolobus*		
• Petroleum Benzene	2.459	9.836
• Chloroform	1.927	7.708
• Methanol	1.461	5.844
6) *Sauropus androgynus*		
• Petroleum Benzene	2.409	9.636
• Chloroform	1.860	7.440
• Methanol	1.577	6.308

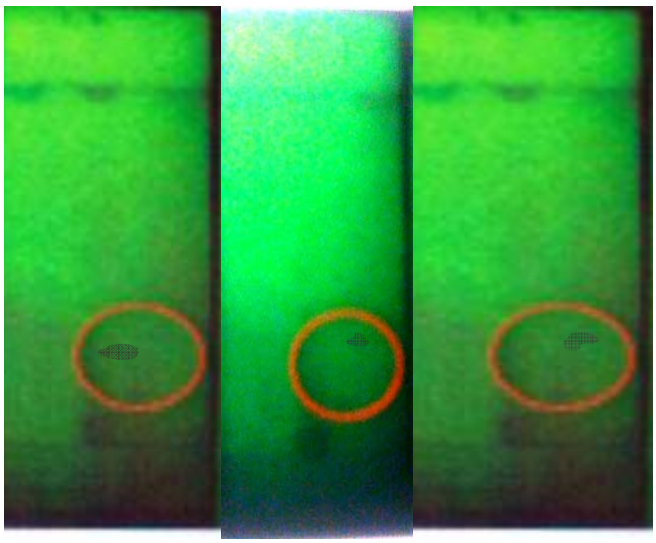
As shown in Table 4.39, all 18 extracts of the selected local vegetables hydroperoxide level ranges between 0.329 nmol to 3.079 nmol. Out of the 18 crude extracts, methanol extract of sweet potato leaves showed highest hydroperoxide value of 3.079 nmol whereas methanol extract of celery showed lowest hydroperoxide value of 0.329 nmol. In comparison to the lipid hydroperoxide standard, the lipid hydroperoxide values of these extracts are at satisfactory level.

4.6 Thin layer Chromatography

Visualizations using thin layer chromatography were carried out to screen bioactive compounds present in the active crude extracts. Those evaluated here were crude chloroform extract of *Murraya koenigii* (L.) which showed positive and good results in 4 of the assays conducted namely DPPH assay, reducing power assay, metal chelating assay and haemolysate catalytic assay. Also evaluated were crude water extract of *Psophocarpus tetragonolobus* which showed good radical scavenging activity in the DPPH assay and good H_2O_2 - reducing activity in the haemolysate catalytic assay, crude water extracts of *Apium graveolens* (L.) which showed good radical scavenging activity in the DPPH assay and good metal chelating activity in the metal chelating assay and crude petroleum benzene extract of *Murraya koenigii* (L.) which showed good radical scavenging activity in the DPPH assay and good reducing activity in the reducing power assay. Short wavelength UV light, long wavelength UV light, iodine vapor, sulphuric acid visualisation and Dragendorff's test were used to observe the bioactive compounds present in these active crude extracts.

4.6.1 Short wavelength visualization

Developed and dried TLC plates were subjected to short wavelength UV light to observe the spots. Only one spot was present in crude chloroform extract of *Murraya koenigii* (L.), crude water extract of *Psophocarpus tetragonolobus* and crude water extracts of *Apium graveolens* (L.) (Figure 4.32) indicating the presence of organic compound in these extracts and it is proven that UV light absorbs aromatic residues making them visible. There were no spots observed in the crude petroleum benzene of *Murraya koenigii* (L.).



(a) (b) (c)

Figure 4.32: TLC plate under short wavelength visualization for (a) crude chloroform extract of *Murraya koenigii (L.*), (b) crude water extract of *Psophocarpus tetragonolobus*, and (c) crude water extracts of *Apium graveolens* (L.)

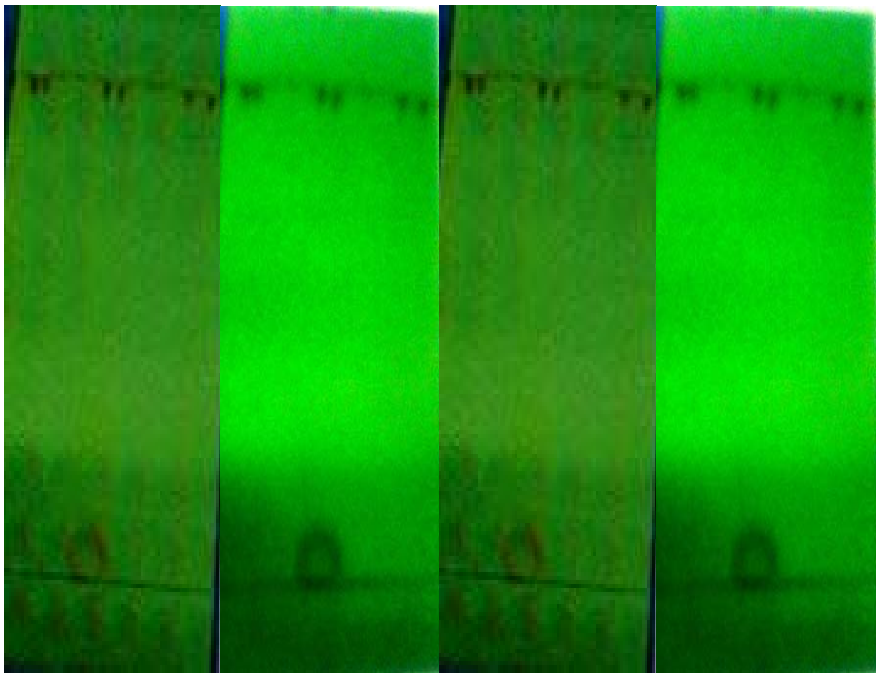
4.6.2 Long wavelength visualization

Figure 4.33 shows that there were no spots present in the TLC plates of all the active crude extract tested, ie crude chloroform extract of *Murraya koenigii* (L.), crude water extract of *Psophocarpus tetragonolobus*, crude water extracts of *Apium graveolens* (L.) and crude petroleum benzene of *Murraya koenigii* (L.).

Figure 4.33: TLC plate under long wavelength visualization for crude chloroform extract of *Murraya koenigii* (L.), crude water extract of *Psophocarpus tetragonolobus*, crude water extracts of *Apium graveolens* (L.) and crude petroleum benzene of *Murraya koenigii* (L.)

4.6.3 Iodine vapour visualization

Figure 4.34 shows that only one spot was present in the crude chloroform extract of *Murraya koenigii* (L.), crude water extract of *Psophocarpus tetragonolobus* and crude water extracts of *Apium graveolens* (L.) and crude petroleum benzene of *Murraya koenigii* (L.) indicating the presence of unsaturared carbon bonds in these extracts.



(a) (b) (c) (d)

Figure 4.34: TLC plate for (a) crude petroleum benzene of *Murraya koenigii* (L.) (b) crude water extract of *Psophocarpus tetragonolobus* (c) crude water extracts of *Apium graveolens* (L.) and, (d) crude petroleum benzene of *Murraya koenigii* (L.) under Iodine vapour visualization

4.6.4 Sulphuric acid visualization

The developed and dry TLC plates sprayed with sulphuric acid showed pink spots present on the plate with petroleum benzene crude extract of *Murraya koenigii* (L.) (Figure 4.35) which indicates the presence of oxidizable components.

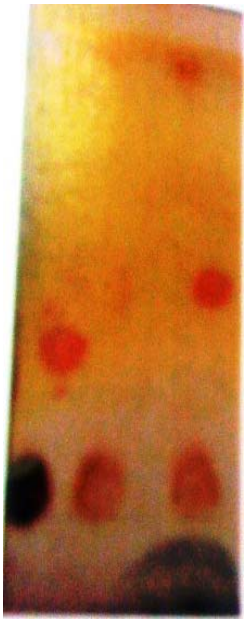


Figure 4.35: TLC plate under sulphuric acid visualization for petroleum benzene crude extract of *Murraya koenigii* (L.)

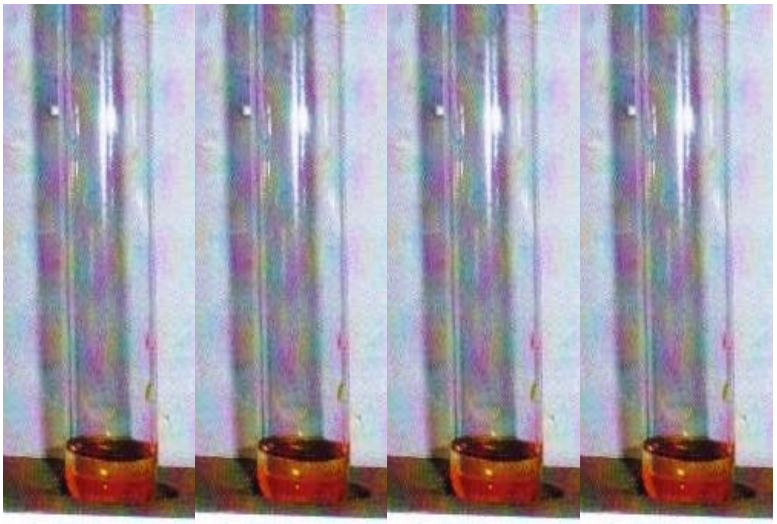
The R_f value of the spots observed under UV shortwave, iodine vapour and sulphuric acid visualization are shown in Table 4.40.

Table 4.40: The R_f value of each spot observed

Sample of Crude extracts	UV Shortwave	Iodine Vapour	Sulphuric Acid
Crude chloroform extract of *Murraya koenigii* (L.)	0.375	0.375	-
Crude Petroleum benzene extract of *Murraya koenigii* (L.)	-	0.377	0.725
Crude water extract of *Apium graveolens* (L.)	0.381	0.379	0.475
Crude water extract of *Psophocarpus tetragonolobus*	0.385	0.376	-

4.6.5 Dragendorff's test

Dragendorff's reagent was added to each selected sample and the changes in colour and formation of a deposit was observed. An orange colour deposit in all the four active crude extracts indicates the presence of alkaloids species (Figure 4.36).



(a) (b) (c) (d)

Figure 4.36: Dragendorff's Test for
(a) crude petroleum benzene of *Murraya koenigii* (L.)
(b) crude water extract of *Psophocarpus tetragonolobus*
(c) crude water extracts of *Apium graveolens* (L.) and,
(d) crude petroleum benzene of *Murraya koenigii* (L.)