

Appendix A: Preparation of Media and Chemicals

Preparation of Malt Extract Agar (MEA)

Malt Extract Agar (MEA) weighing 13.44 g was dissolved in 400 ml of distilled water in an Erlenmeyer flask using a magnetic stirrer. The solution was poured into universal bottles until half full, then autoclaved at 121°C for 20 minutes.

Preparation of Glucose-Yeast-Malt-Peptone (GYMP) agar

A 500 ml Erlenmeyer flask was filled with 0.4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 g NH_4Cl , 0.4 g KH_2PO_4 , 0.4 g K_2HPO_4 , 6.0 g glucose, 3.2 g peptone, 3.2 g malt extract and 3.2 g yeast extract and 7.0 g agar. The contents were dissolved in 400 ml of distilled water using a magnetic stirrer. The flask was covered with non-absorbent cotton plugs and aluminium foil, then autoclaved at 121°C for 20 minutes. The sterilized medium was left to cool, then poured into Petri dishes aseptically and allowed to harden.

Precaution:

Media which is too hot should not be poured into the Petri dishes because condensed water can fall on to the agar. If a lot of condensed water forms on the lid, incubate the plates upside down; the inoculum sticks to the agar and are not dislodged.

Preparation of Glucose-Yeast-Malt-Peptone (GYMP) liquid media

A 500 ml Erlenmeyer flask was filled with 0.4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 g NH_4Cl , 0.4 g KH_2PO_4 , 0.4 g K_2HPO_4 , 6.0 g glucose, 3.2 g peptone, 3.2 g malt extract and 3.2 g yeast extract. The contents were dissolved in 400 ml of distilled water using a magnetic stirrer. The flask was covered with non-absorbent cotton plugs and aluminium foil, then autoclaved at 121°C for 20 minutes. The sterilized medium was left to cool.

Preparation of Basic Medium 199

One sachet of medium 199 (Flow Lab, Australia) containing Earle's salt with L-Glutamine and HEPES (N-2-Hydroxyethyl-Piperazine-N-2-Ethane-Sulfonoc Acid, Sigma, USA) without sodium bicarbonate (Flow Lab.) was made up to 1 litre with distilled water. Two grams of sodium bicarbonate (NaHCO₃, Merck, Germany) was added to the medium. The medium was filter sterilized using a 0.22 µm filter membrane (Schleicher & Schuell) and stored at 4°C for up to 4 months.

Preparation of Basic RPMI 1640 Medium

Medium was prepared by dissolving 10.39 g of RPMI 1640 powder (Sigma) and 2.0 g of sodium bicarbonate in 1 litre of distilled water. The pH of the medium was calibrated to pH 7.4 (Hanna Instruments 8417). The medium was then filter sterilized using a 0.22 µm filter membrane (Schleicher & Schuell) into sterile bottles and kept at 4°C.

10% Supplemented Medium 199 and RPMI 1640 Medium

100 ml of 10% supplemented medium 199 and RPMI 1640 were prepared using 90 ml of basic medium, supplemented with 10 ml inactivated Foetal Calf Serum (FCS, PAA Lab. Austria), 1 ml (100 µg/ml) and 1 ml (100 IU/ml) of streptomycin and penicillin (PAA Lab Austria) respectively and 1 ml of fungizone (PAA Lab Austria). The media was filter sterilized using a 0.22 µm filter membrane and stored at 4°C for up to 2 weeks.

20% Supplemented Medium 199 and RPMI 1640 Medium

50 ml of 20% supplemented medium 199 or RPMI 1640 was prepared using a 45 ml of 10% supplemented medium was added with 5 ml inactivated FCS. The medium was

filter sterilized using a 0.22 µm filter membrane and stored at 4°C for up to 2 weeks. This 20% supplemented medium was used to revive cells.

Phosphate Buffered Saline (PBS) pH 7.2

The phosphate buffered saline (PBS) was prepared using 1.52 g of sodium phosphate anhydrous (Na₂HPO₄, BDH Lab Supplies), 0.58 g of potassium dihydrogen orthophosphate (KH₂PO₄, Merck) and 8.5 g of sodium chloride (NaCl, BDH Lab Supplies), that were dissolved in sterile distilled water and the volume was made up to 1 litre. The pH of the buffer was adjusted to 7.2 using a pH meter (Hanna Instruments). The buffer was then filtered using a 0.22 µm filter membrane (Whatman 541) and stored at room temperature. The solution was autoclaved (P-Selecta) and kept at room temperature.

10% Dimethylsulfoxide (DMSO)

The 10% DMSO solution was prepared by mixing 99.9% dimethylsulfoxide DMSO (Sigma) with sterile distilled water in a ration of 1:9. Freshly prepared 10% DMSO was used for the neutral red cytotoxic assay.

0.4 % Tryphan blue

0.4% Tryphan blue was prepared by dissolving 0.2 g of powdered tryphan blue (Sigma) in 50 ml of sterile water. The solution was kept at room temperature.

Neutral Red Cytotoxicity Activity Assay

Preparation of Solutions

Neutral Red Stock Solution

0.4 g of Neutral Red (ICN Biomedicals Inc, USA) was dissolved in 100 ml sterile distilled water. The stock solution was wrapped with aluminium foil and stored at 4°C.

Neutral Red Medium

The Neutral Red stock solution was diluted (1:80) in treatment culture medium to give a final concentration of 50 µg/ml. Prepared Neutral Red medium were incubated overnight at room temperature in the dark. This solution was centrifuged twice at 1500 rpm for 10 minutes before any use to remove any fine needle-like precipitate of dye crystals.

Neutral Red Washing Solution

10% of calcium chloride (Sigma) was dissolved in 1 ml formaldehyde (Sigma) and 89 ml of distilled water. The solution was kept at 4°C.

Neutral Red Resorb Solution

1 ml of glacial acetic acid (BDH Lab Supplies) was dissolved in 50 ml of absolute ethanol (Hamburg Chemicals) and 49 ml of distilled water. The solution was kept at 4°C.

Antioxidant Activity Assay

DPPH Free Radical Scavenging Activity Assay

Preparation of stock solution

A stock solution of 50 mg/ml of each extract was prepared and wrapped in aluminium foil. The crude dichloromethane extracts were dissolved in 1 ml methanol (System)

Preparation of Ascorbic Acid

A stock of ascorbic acid (Sigma) in methanol was prepared at concentration of 400 µg/ml. The stock solution was kept in flask wrapped with aluminium foil.

Preparation of DPPH

A stock of DPPH (Sigma) in methanol was prepared at concentration of 8 mg/ml. The stock solution was kept in flask wrapped with aluminium foil.

Reducing Power Assay

Preparation of (10 mg/ml) Potassium Ferricyanide 1% solution

0.1 g $K_3Fe(CN)_6$ (System) was dissolved in 10 ml of distilled water in a centrifuge tube wrapped with aluminum foil. The tube was then vortex.

Preparation of (100mg/ml) Trichloroacetic acid (TCA) 10%

1 g $C_2HCl_3O_2$ (Sigma) was dissolved in 10 ml of distilled water in a centrifuge tube wrapped with aluminum foil. The tube was then vortex.

Preparation of (1 mg/ml) Ferric chloride solution 0.1%

0.01 g of ferric chloride (BDH, Lab Supplies) was dissolved in 10 ml of distilled water in a centrifuge tube wrapped with aluminum foil. The tube was then vortex.

Preparation of 0.4 M Monobasic stock

0.4 M monobasic stock solution was prepared by dissolving 27.8 g of sodium phosphate monobasic Na_2HPO_4 in 500 ml of distilled water. The stock solution was kept in a sterile bottle wrapped with aluminium foil at room temperature

Preparation of 0.4 M Dibasic Stock

0.4 M dibasic stock solution was prepared by dissolving 53.65g of sodium phosphate dibasic (heptahydrate) $\text{Na}_2\text{H}_2\text{PO}_4$ or 28.4 g of the anhydrous form in 500 ml of distilled water. The stock solution was kept in a sterile bottle wrapped with aluminium foil at room temperature.

Preparation of 0.2 M Buffer (Phosphate Buffer)

Combine 600 ml distilled water and the appropriate amounts of monobasic and dibasic stock solution to obtain the desired pH of 6.6. the stock solution was kept in a sterile bottle wrapped with aluminium foil.

Metal Chelating Assay

Preparation of positive control EDTA

1 g of EDTA was dissolved in 4 ml of distilled water in a centrifuge tube wrapped with aluminum foil. The tube was then vortex

10 ml of methanol was dissolved with 0.0246 g of 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt ($\text{C}_{20}\text{H}_{13}\text{N}_4\text{NaO}_6\text{S}_2$) that was purchased from Sigma Aldrich. The tube was wrapped in aluminium foil and vortex.

Appendix B: Experimental and Statistical Data

1. Antioxidant activity of *Marasmius* spp. crude extracts using DPPH radical scavenging assay.

1.1 *Marasmius ruforotula* KUM 20111

a. Absorbance at 515nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval.

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.639	0.628	0.635	0.634	3.206	0.005
1	0.635	0.622	0.629	0.629	4.020	0.005
2	0.631	0.617	0.624	0.624	4.733	0.006
15	0.617	0.603	0.609	0.610	6.921	0.006
30	0.612	0.595	0.604	0.604	7.837	0.007
45	0.609	0.591	0.599	0.600	8.448	0.007
60	0.608	0.590	0.596	0.598	8.702	0.007
75	0.594	0.588	0.610	0.597	8.804	0.009

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.614	0.634	0.615	0.621	5.191	0.009
1	0.600	0.619	0.601	0.607	7.379	0.009
2	0.590	0.610	0.593	0.598	8.753	0.009
15	0.554	0.571	0.561	0.562	14.198	0.007
30	0.533	0.549	0.546	0.543	17.150	0.007
45	0.525	0.537	0.533	0.532	18.830	0.005
60	0.519	0.527	0.524	0.523	20.102	0.003
75	0.509	0.522	0.515	0.515	21.323	0.005

50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.613	0.616	0.620	0.616	5.903	0.003
1	0.600	0.607	0.607	0.605	7.684	0.003
2	0.584	0.595	0.591	0.590	9.924	0.005
15	0.522	0.546	0.529	0.532	18.728	0.010
30	0.492	0.524	0.503	0.506	22.697	0.013
45	0.473	0.509	0.484	0.489	25.394	0.015
60	0.453	0.495	0.460	0.469	28.346	0.018
75	0.440	0.485	0.454	0.460	29.822	0.019

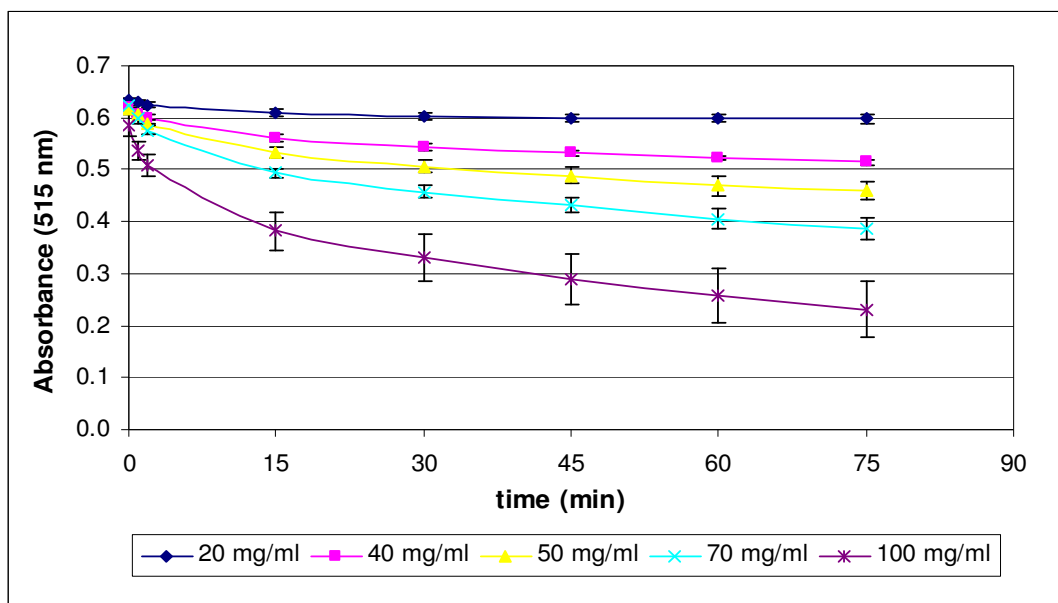
70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.632	0.611	0.622	0.622	5.089	0.009
1	0.611	0.588	0.597	0.599	8.601	0.009
2	0.588	0.566	0.574	0.576	12.061	0.009
15	0.506	0.492	0.484	0.494	24.580	0.009
30	0.471	0.457	0.442	0.457	30.280	0.012
45	0.447	0.434	0.412	0.431	34.198	0.014
60	0.424	0.411	0.382	0.406	38.066	0.018
75	0.406	0.394	0.360	0.387	40.967	0.019

100 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.557	0.594	0.603	0.585	10.738	0.020
1	0.513	0.550	0.550	0.538	17.913	0.017
2	0.480	0.527	0.522	0.510	22.188	0.021
15	0.331	0.414	0.403	0.383	41.578	0.037
30	0.269	0.368	0.355	0.331	49.517	0.044
45	0.222	0.331	0.317	0.290	55.725	0.048
60	0.186	0.303	0.287	0.259	60.509	0.052
75	0.154	0.277	0.261	0.231	64.784	0.055

b. Graph of the mean of absorbance at 515 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

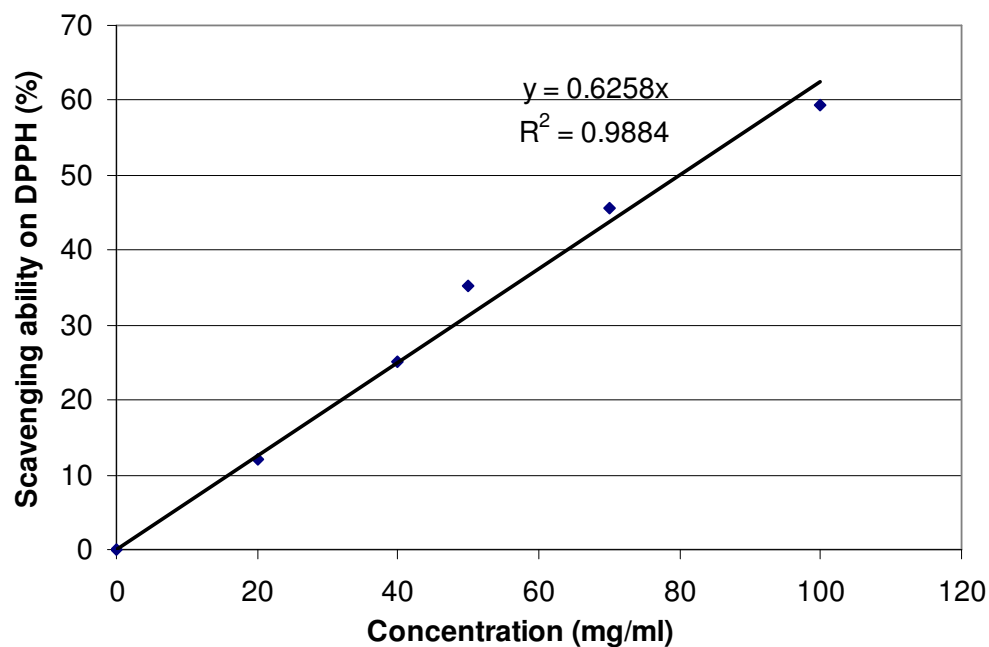
c. ANOVA test for *Marasmius ruforotula* KUM 20111: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.036	*0.098	*0.147	*0.216	*0.326
1	*0.031	*0.083	*0.135	*0.193	*0.279
2	*0.026	*0.074	*0.121	*0.170	*0.251
15	0.011	*0.039	*0.063	*0.088	*0.124
30	0.006	*0.019	*0.037	*0.051	0.072
45	0.002	0.008	*0.053	0.025	0.031
#60	0.001	0.008	0.009	0.019	0.067
75	0.000	0.000	0.000	0.000	0.000
P-value	0.0001	0.000	0.000	0.000	0.000

*Denotes the mean differences were significant (P<0.05, ANOVA test)

#Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

e. Scavenging activity of *M. ruforotula* KUM 20111 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



d. Percentage of DPPH· radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	6.75	7.90	11.46	8.702	2.00
40	18.95	19.66	21.70	20.10	1.17
50	26.77	28.48	29.79	28.35	1.24
70	38.66	39.77	35.77	38.07	1.69
100	57.98	61.26	62.29	60.51	1.84

2.1 *Marasmius ruforotula* KUM 20112

a. Absorbance at 515nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration of stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.630	0.623	0.623	0.625	4.820	0.003
1	0.621	0.615	0.616	0.617	6.038	0.003
2	0.619	0.613	0.612	0.615	6.443	0.003
15	0.604	0.593	0.595	0.597	9.082	0.005
30	0.599	0.587	0.59	0.592	9.893	0.005
45	0.594	0.581	0.584	0.586	10.756	0.006
60	0.592	0.578	0.582	0.584	11.111	0.006
75	0.591	0.575	0.578	0.581	11.517	0.007

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.627	0.612	0.612	0.617	6.088	0.007
1	0.605	0.595	0.587	0.596	9.335	0.007
2	0.597	0.589	0.576	0.587	10.604	0.009
15	0.542	0.549	0.515	0.535	18.519	0.015
30	0.523	0.536	0.486	0.515	21.613	0.021
45	0.502	0.524	0.457	0.494	24.759	0.028
60	0.493	0.519	0.438	0.483	26.433	0.034
75	0.480	0.508	0.416	0.468	28.767	0.039

50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.578	0.601	0.606	0.595	9.437	0.012
1	0.583	0.592	0.593	0.589	10.299	0.004
2	0.576	0.584	0.585	0.582	11.466	0.004
15	0.522	0.534	0.535	0.530	19.280	0.006
30	0.498	0.512	0.513	0.508	22.730	0.007
45	0.480	0.497	0.499	0.492	25.114	0.009
60	0.468	0.485	0.488	0.480	26.890	0.009
75	0.455	0.474	0.476	0.468	28.716	0.009

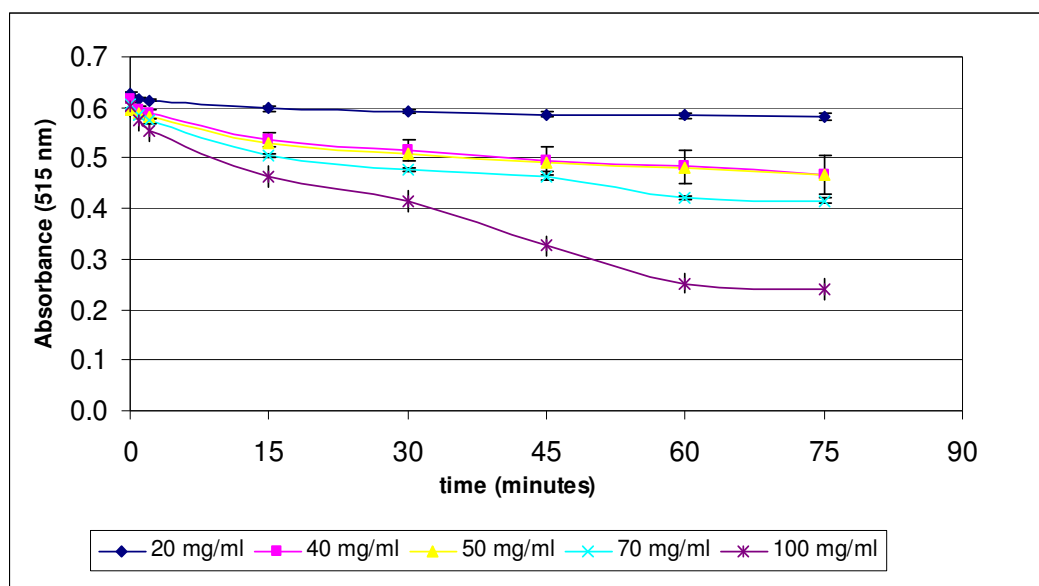
70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.598	0.609	0.616	0.608	7.509	0.007
1	0.578	0.586	0.590	0.585	11.010	0.005
2	0.566	0.575	0.578	0.573	12.785	0.005
15	0.499	0.505	0.510	0.505	23.186	0.004
30	0.471	0.477	0.481	0.476	27.499	0.004
45	0.474	0.468	0.451	0.464	29.325	0.010
60	0.421	0.425	0.417	0.421	35.921	0.003
75	0.411	0.423	0.413	0.416	36.733	0.005

100 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.574	0.579	0.652	0.602	8.422	0.036
1	0.543	0.551	0.630	0.575	12.532	0.039
2	0.521	0.530	0.611	0.554	15.677	0.040
15	0.417	0.434	0.539	0.463	29.477	0.054
30	0.362	0.379	0.504	0.415	36.834	0.063
45	0.323	0.322	0.332	0.326	50.431	0.004
60	0.298	0.225	0.234	0.252	61.593	0.032
75	0.244	0.232	0.245	0.240	63.420	0.006

b. Graph of the mean of absorbance at 515 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius ruforotula* KUM 20112: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.041	*0.134	*0.115	*0.187	*0.264
1	*0.033	*0.112	*0.109	*0.164	*0.237
2	*0.031	*0.104	*0.101	*0.152	*0.216
15	*0.013	*0.052	*0.05	*0.084	*0.126
30	0.008	0.032	*0.027	*0.055	0.077
45	0.002	0.011	0.012	*0.043	0.028
#60	0.003	0.015	0.012	0.005	0.035
75	0.000	0.000	0.000	0.000	0.000
P-value	0.000	0.000	0.000	0.000	0.000

*Denotes the mean differences were significant (P<0.05, ANOVA test)

40 mg/ml

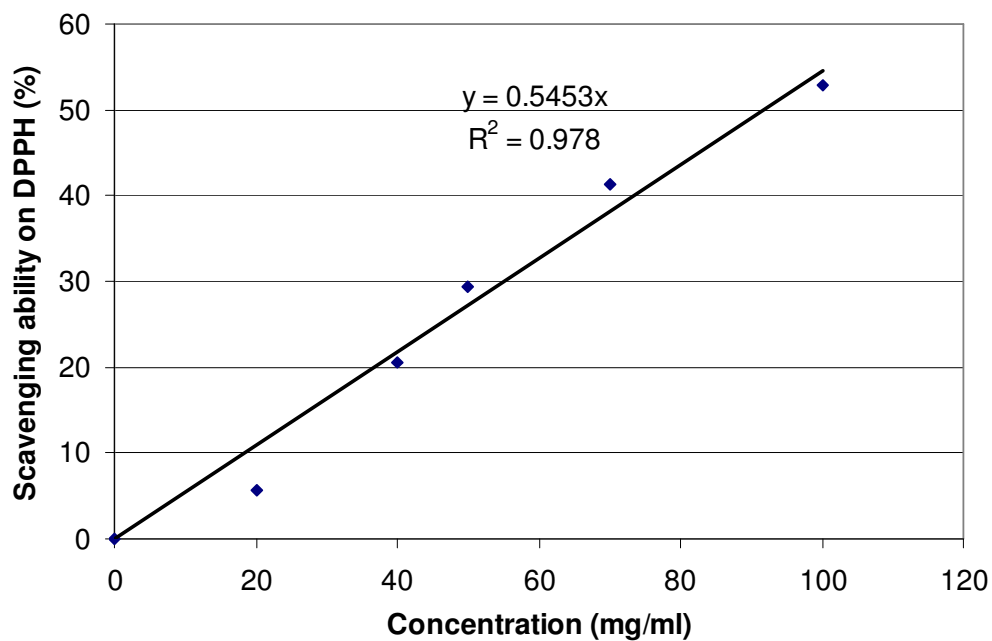
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.580	0.560	0.559	0.566	14.58	0.010
1	0.568	0.548	0.546	0.554	16.44	0.010
2	0.563	0.542	0.539	0.548	17.35	0.011
15	0.524	0.506	0.500	0.510	23.08	0.010
30	0.500	0.488	0.485	0.491	25.94	0.006
45	0.489	0.473	0.463	0.475	28.36	0.011
60	0.476	0.458	0.447	0.460	30.57	0.012
75	0.462	0.443	0.432	0.446	32.78	0.012

#Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	10.98	10.54	11.81	11.11	0.53
40	27.76	26.85	26.06	26.89	0.70
50	29.86	30.97	30.18	30.34	0.47
70	38.32	34.57	34.88	35.92	1.70
100	62.15	63.43	62.85	62.81	0.52

e. Scavenging activity of *M. ruforotula* KUM 20112 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2.2 *Marasmius guyanensis* KUM 20117

a. Absorbance at 517 nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.601	0.609	0.597	0.602	9.15	0.005
1	0.595	0.602	0.591	0.596	10.11	0.005
2	0.591	0.599	0.587	0.592	10.66	0.005
15	0.570	0.579	0.570	0.573	13.57	0.004
30	0.562	0.574	0.564	0.567	14.53	0.005
45	0.557	0.567	0.556	0.560	15.54	0.005
60	0.553	0.559	0.551	0.554	16.39	0.003
75	0.549	0.556	0.548	0.551	16.91	0.004

50 mg/ml

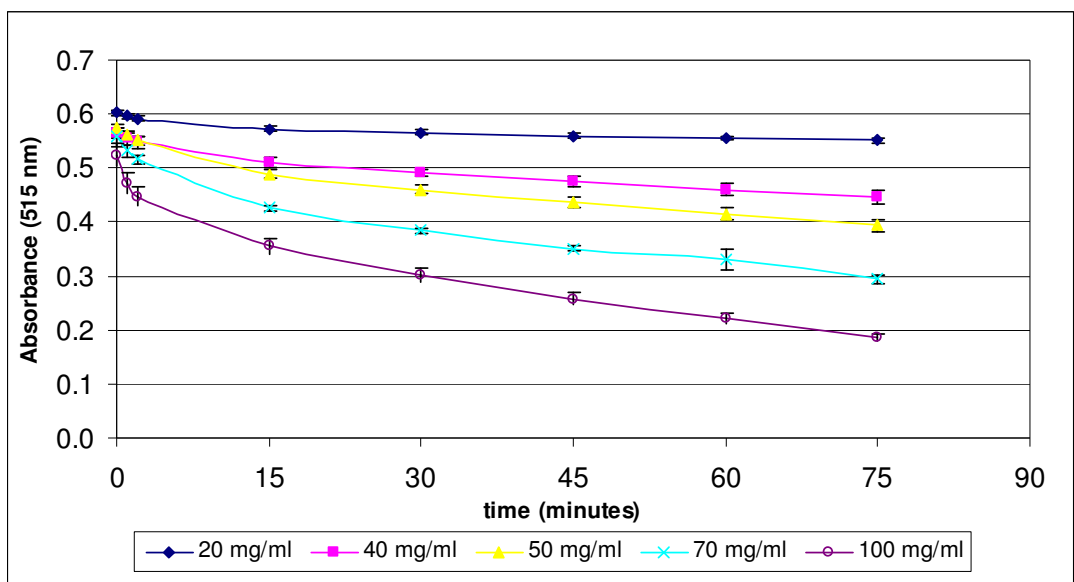
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.568	0.570	0.584	0.574	13.42	0.007
1	0.555	0.556	0.573	0.561	15.33	0.008
2	0.545	0.547	0.563	0.552	16.79	0.008
15	0.480	0.486	0.501	0.489	26.24	0.009
30	0.450	0.459	0.473	0.461	30.52	0.009
45	0.425	0.436	0.450	0.437	34.09	0.010
60	0.404	0.413	0.430	0.416	37.31	0.011
75	0.381	0.393	0.408	0.394	40.57	0.011

70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.536	0.560	0.576	0.557	15.94	0.016
1	0.517	0.535	0.543	0.532	19.81	0.011
2	0.503	0.519	0.525	0.516	22.22	0.009
15	0.419	0.430	0.428	0.426	35.80	0.005
30	0.380	0.389	0.383	0.384	42.08	0.004
45	0.348	0.357	0.347	0.351	47.11	0.004
60	0.322	0.359	0.315	0.332	49.92	0.019
75	0.296	0.302	0.285	0.294	55.61	0.007

100 mg/ml						
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.502	0.528	0.545	0.525	20.81	0.018
1	0.462	0.478	0.477	0.472	28.76	0.007
2	0.435	0.454	0.451	0.447	32.63	0.008
15	0.337	0.367	0.361	0.355	46.46	0.013
30	0.281	0.315	0.309	0.302	54.50	0.015
45	0.233	0.275	0.266	0.258	61.09	0.018
60	0.297	0.139	0.230	0.222	66.52	0.065
75	0.160	0.205	0.195	0.187	71.85	0.019

b. Graph of the mean of absorbance at 515 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius guyanensis*. **KUM 20117**: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.048	*0.106	*0.165	*0.225	*0.303
1	*0.042	*0.094	*0.152	*0.200	*0.250
2	*0.038	*0.088	*0.143	*0.184	*0.225
15	*0.019	*0.050	*0.080	*0.094	*0.133
30	*0.012	*0.031	*0.052	*0.052	*0.080
45	0.006	0.015	*0.028	0.019	0.036
#60	0.003	0.015	0.015	0.008	0.035
75	0.000	0.000	0.000	0.000	0.000

P-value	0.000	0.000	0.000	0.000	0.000
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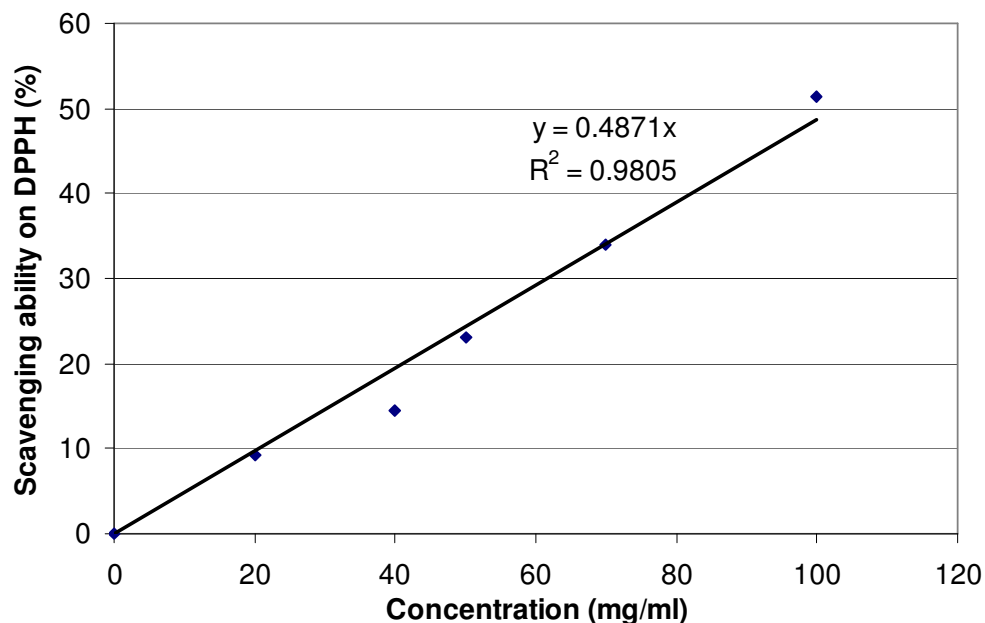
*Denotes the mean differences were significant (P<0.05, ANOVA test)

Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	16.01	16.46	16.70	16.39	0.28
40	30.23	31.01	30.47	30.57	0.33
50	38.14	36.98	36.80	37.31	0.59
70	50.87	53.05	50.38	51.43	1.16
100	66.50	66.32	66.73	66.52	0.17

e. Scavenging activity of *M. guyanensis* KUM 20117 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements (n = 3).



2.3 *Marasmius guyanensis* KUM 20044

a. Absorbance at 515nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration of stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.664	0.669	0.674	0.669	3.463	0.004
1	0.657	0.660	0.650	0.656	5.387	0.004
2	0.651	0.654	0.660	0.655	5.483	0.004
15	0.625	0.625	0.632	0.627	9.476	0.003
30	0.608	0.606	0.613	0.609	12.121	0.003
45	0.598	0.597	0.602	0.599	13.564	0.002
60	0.580	0.589	0.595	0.588	15.152	0.006
75	0.583	0.578	0.587	0.583	15.921	0.004

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.689	0.689	0.683	0.687	0.866	0.003
1	0.672	0.663	0.686	0.674	2.790	0.009
2	0.661	0.652	0.675	0.663	4.377	0.009
15	0.602	0.588	0.611	0.600	13.372	0.009
30	0.558	0.548	0.571	0.559	19.336	0.009
45	0.531	0.520	0.544	0.532	23.280	0.010
60	0.510	0.498	0.523	0.510	26.359	0.010
75	0.491	0.479	0.504	0.491	29.101	0.010

50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.691	0.692	0.682	0.688	0.673	0.004
1	0.689	0.687	0.666	0.681	1.780	0.010
2	0.672	0.672	0.652	0.665	3.992	0.009
15	0.560	0.573	0.563	0.565	18.422	0.006
30	0.502	0.521	0.513	0.512	26.118	0.008
45	0.461	0.484	0.481	0.475	31.409	0.010
60	0.429	0.455	0.454	0.446	35.642	0.012
75	0.453	0.482	0.423	0.453	34.680	0.024

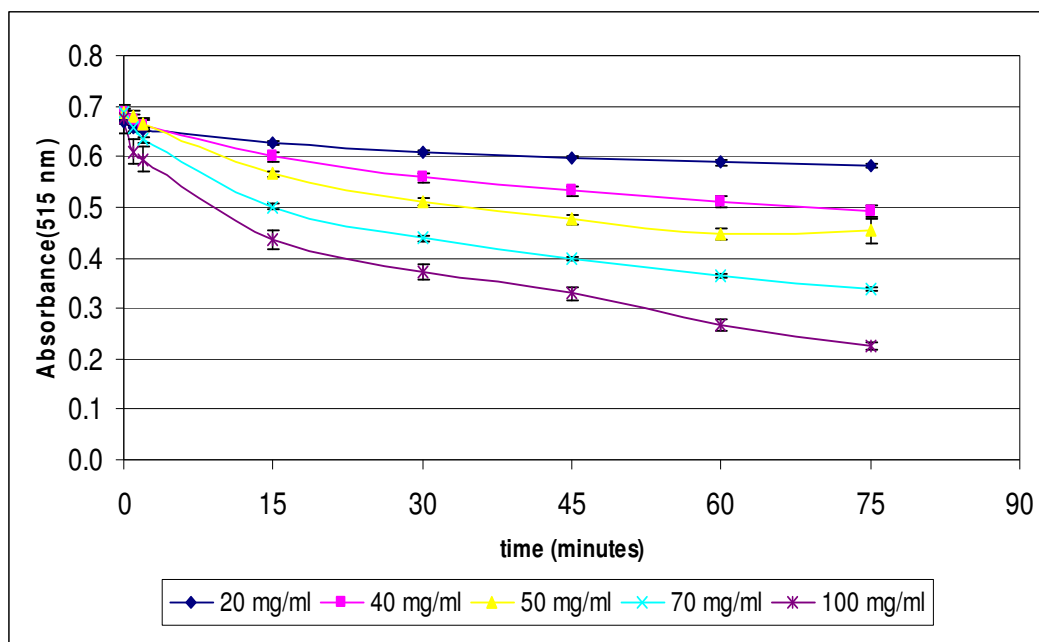
70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.676	0.692	0.692	0.687	0.914	0.008
1	0.647	0.666	0.657	0.657	5.243	0.008
2	0.624	0.640	0.635	0.633	8.658	0.007
15	0.493	0.506	0.502	0.500	27.802	0.005
30	0.431	0.443	0.439	0.438	36.845	0.005
45	0.391	0.402	0.400	0.398	42.617	0.005
60	0.359	0.369	0.366	0.365	47.379	0.004
75	0.333	0.341	0.337	0.337	51.371	0.003

100 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.681	0.662	0.681	0.675	2.646	0.009
1	0.624	0.602	0.604	0.610	11.977	0.010
2	0.592	0.573	0.619	0.595	14.190	0.019
15	0.412	0.394	0.501	0.436	37.133	0.047
30	0.338	0.324	0.455	0.372	46.272	0.059
45	0.289	0.277	0.423	0.330	52.429	0.066
60	0.216	0.206	0.375	0.266	61.664	0.077
75	0.205	0.216	0.254	0.225	67.532	0.021

b. Graph of the mean of absorbance at 517 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius guyanensis* **KUM 20044**: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.081	*0.177	*0.219	*0.346	*0.379
1	*0.068	*0.163	*0.211	*0.316	*0.314
2	*0.067	*0.152	*0.196	*0.293	*0.299
15	*0.039	*0.090	*0.096	*0.160	*0.140
30	*0.021	*0.049	*0.043	*0.097	0.077
45	*0.011	*0.021	0.006	*0.057	0.034
#60	0.005	0.019	0.017	0.010	0.030
75	0.000	0.000	0.000	0.000	0.000
P-value	0.000	0.000	0.000	0.000	0.000

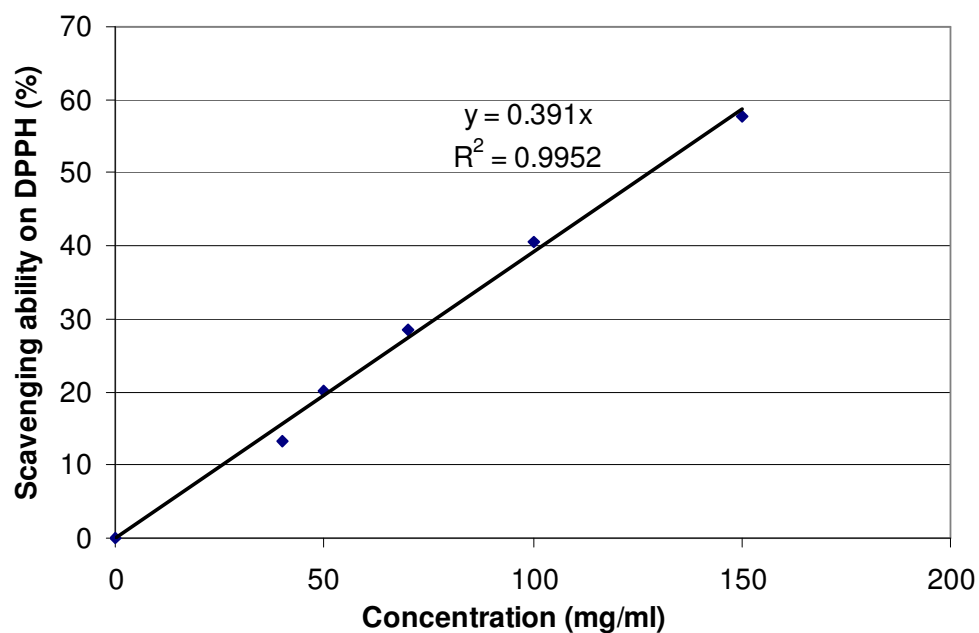
*Denotes the mean differences were significant ($P < 0.05$, ANOVA test)

#Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	14.48	13.75	15.78	14.67	0.84
40	25.97	27.41	25.7	26.36	0.75
50	35.24	34.76	36.92	35.64	0.93
70	57.38	57.74	56.9	57.34	0.34
100	68.59	66.74	64.98	66.77	1.47

e. Scavenging activity of *M. guyanensis* KUM 20044 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2.5 *Marasmius guyanensis* KUM 20222

a. Absorbance at 517 nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.504	0.535	0.512	0.517	5.657	0.013
1	0.494	0.523	0.501	0.506	7.664	0.012
2	0.490	0.515	0.495	0.500	8.759	0.011
15	0.453	0.456	0.441	0.450	17.883	0.006
30	0.440	0.433	0.417	0.430	21.533	0.010
45	0.432	0.416	0.398	0.415	24.209	0.014
60	0.424	0.402	0.385	0.404	26.338	0.016
75	0.418	0.390	0.372	0.393	28.224	0.019

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.508	0.505	0.560	0.524	4.319	0.025
1	0.487	0.482	0.534	0.501	8.577	0.023
2	0.479	0.471	0.523	0.491	10.401	0.023
15	0.398	0.384	0.435	0.406	25.973	0.022
30	0.365	0.348	0.398	0.370	32.421	0.021
45	0.339	0.320	0.369	0.343	37.470	0.020
60	0.319	0.298	0.348	0.322	41.302	0.020
75	0.301	0.279	0.328	0.303	44.769	0.020

50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.490	0.494	0.475	0.486	11.253	0.008
1	0.474	0.478	0.455	0.469	14.416	0.010
2	0.457	0.460	0.436	0.451	17.701	0.011
15	0.346	0.353	0.325	0.341	37.713	0.012
30	0.298	0.307	0.280	0.295	46.168	0.011
45	0.262	0.272	0.244	0.259	52.676	0.012
60	0.236	0.247	0.219	0.234	57.299	0.012
75	0.215	0.226	0.198	0.213	61.131	0.012

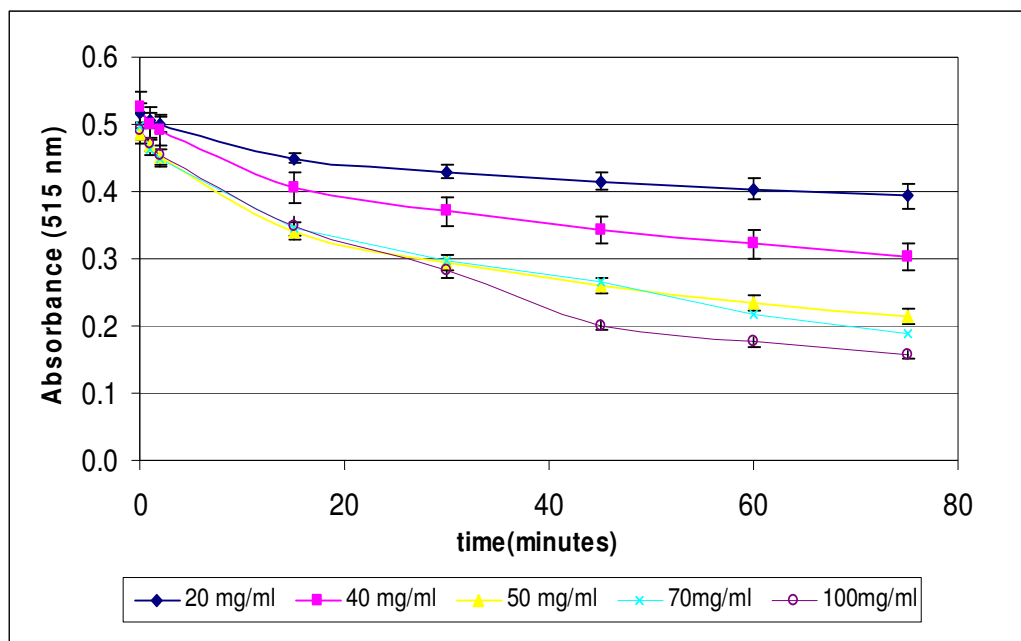
70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.503	0.489	0.497	0.496	9.428	0.006
1	0.474	0.458	0.460	0.464	15.328	0.007
2	0.458	0.442	0.443	0.448	18.309	0.007
15	0.354	0.343	0.343	0.347	36.740	0.005
30	0.303	0.296	0.295	0.298	45.620	0.004
45	0.268	0.264	0.263	0.265	51.642	0.002
60	0.217	0.218	0.215	0.217	60.462	0.001
75	0.201	0.150	0.217	0.189	65.450	0.029

100 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.459	0.517	0.500	0.492	10.219	0.024
1	0.434	0.500	0.484	0.473	13.747	0.028
2	0.411	0.486	0.465	0.454	17.153	0.032
15	0.284	0.399	0.360	0.348	36.557	0.048
30	0.230	0.301	0.316	0.282	48.479	0.038
45	0.190	0.132	0.282	0.201	63.260	0.062
60	0.162	0.111	0.257	0.177	67.762	0.060
75	0.141	0.193	0.136	0.157	71.411	0.026

b. Graph of the mean of absorbance at 517 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius guyanensis* **KUM 20222**: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.113	*0.203	*0.252	*0.315	*0.280
1	*0.102	*0.179	*0.235	*0.296	*0.247
2	*0.096	*0.169	*0.217	*0.277	*0.231
15	*0.046	*0.084	*0.107	*0.171	*0.130
30	0.026	*0.049	*0.061	*0.106	*0.081
45	0.012	0.021	*0.025	0.025	*0.048
#60	0.010	0.019	0.021	0.020	0.002
75	0.000	0.000	0.000	0.000	0.000
P-value	0.000	0.000	0.000	0.000	0.000

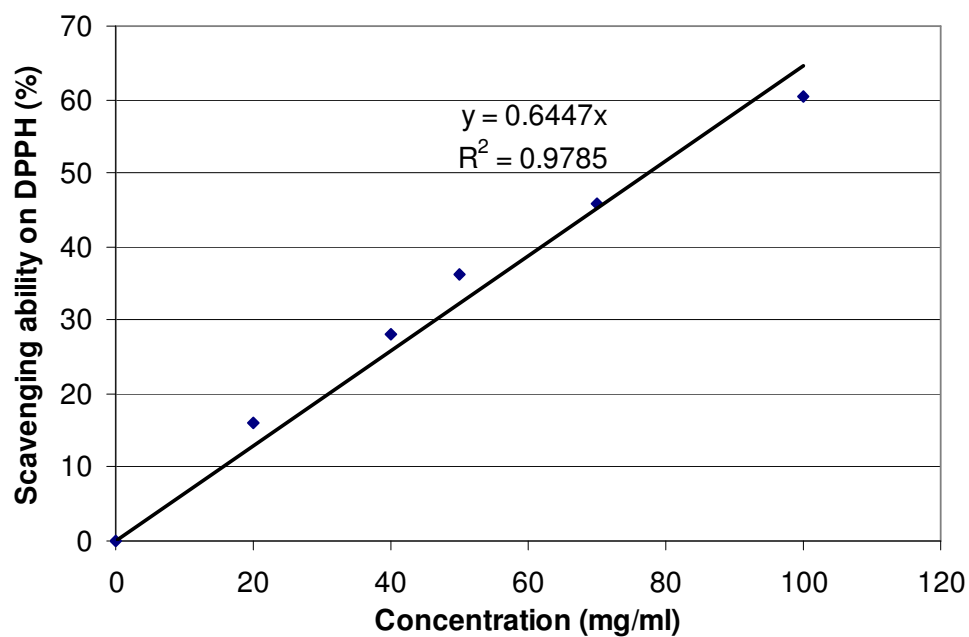
*Denotes the mean differences were significant (P<0.05, ANOVA test).

#Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes.

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	26.31	25.74	26.97	26.34	0.50
40	40.19	39.86	43.85	41.30	1.81
50	57.24	57.32	57.34	57.30	0.04
70	68.53	67.73	67.02	67.76	0.62
100	79.40	79.42	78.21	79.01	0.57

e. Scavenging activity of *M. guyanensis* KUM 20222 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2.6 *Marasmius selangorensis* KUM 20181

- a. Absorbance at 515nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration of stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.655	0.660	0.672	0.662	3.026	0.007
1	0.653	0.657	0.669	0.660	3.416	0.007
2	0.651	0.655	0.668	0.658	3.660	0.007
15	0.643	0.649	0.658	0.650	4.832	0.006
30	0.644	0.649	0.653	0.649	5.027	0.004
45	0.638	0.631	0.633	0.634	7.174	0.003
60	0.543	0.565	0.564	0.557	18.399	0.010
75	0.552	0.551	0.553	0.552	19.180	0.001

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.627	0.650	0.657	0.645	5.612	0.013
1	0.610	0.631	0.650	0.630	7.711	0.016
2	0.601	0.621	0.646	0.623	8.834	0.018
15	0.551	0.574	0.629	0.585	14.397	0.033
30	0.531	0.554	0.623	0.569	16.642	0.039
45	0.518	0.542	0.618	0.559	18.106	0.043
60	0.508	0.534	0.616	0.553	19.082	0.046
75	0.502	0.530	0.518	0.517	24.353	0.011

50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.607	0.644	0.649	0.633	7.272	0.019
1	0.590	0.628	0.629	0.616	9.858	0.018
2	0.575	0.614	0.611	0.600	12.152	0.018
15	0.498	0.542	0.541	0.527	22.840	0.021
30	0.469	0.515	0.514	0.499	26.891	0.021
45	0.449	0.498	0.495	0.481	29.624	0.022
60	0.433	0.484	0.480	0.466	31.820	0.023
75	0.420	0.472	0.467	0.453	33.675	0.023

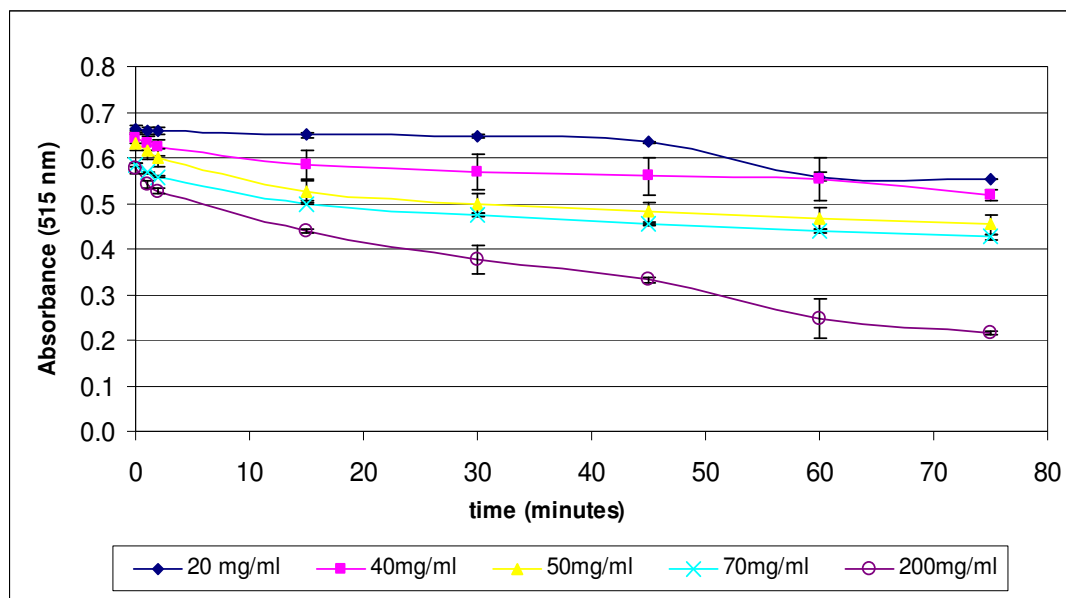
70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.579	0.585	0.588	0.584	14.495	0.004
1	0.566	0.567	0.570	0.568	16.886	0.002
2	0.557	0.557	0.560	0.558	18.302	0.001
15	0.501	0.496	0.502	0.500	26.842	0.003
30	0.479	0.470	0.479	0.476	30.307	0.004
45	0.453	0.455	0.452	0.453	33.626	0.001
60	0.435	0.441	0.440	0.439	35.774	0.003
75	0.429	0.416	0.433	0.426	37.628	0.007

100 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.566	0.571	0.592	0.576	15.617	0.011
1	0.536	0.535	0.550	0.540	20.888	0.007
2	0.522	0.521	0.535	0.526	22.987	0.006
15	0.438	0.434	0.442	0.438	35.871	0.003
30	0.332	0.396	0.401	0.376	44.900	0.031
45	0.324	0.336	0.337	0.332	51.342	0.006
60	0.224	0.211	0.305	0.247	63.885	0.042
75	0.221	0.212	0.211	0.215	68.570	0.004

b. Graph of the mean of absorbance at 517 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

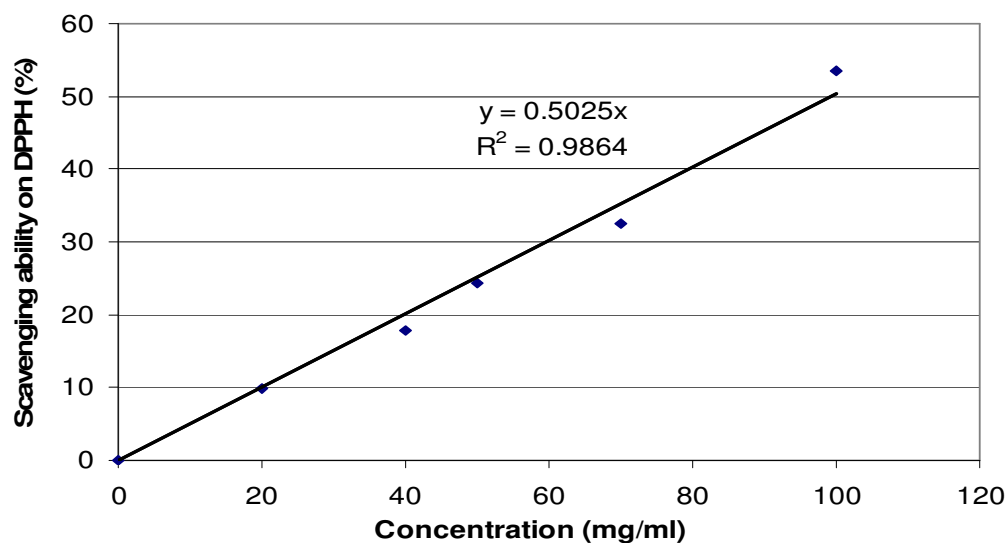
c. ANOVA test for *Marasmius selangorensis* KUM 20181: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.105	*0.092	*0.168	*0.150	*0.330
1	*0.102	*0.078	*0.150	*0.134	*0.294
2	*0.101	0.070	*0.134	*0.124	*0.279
15	*0.093	0.032	*0.061	*0.042	*0.191
30	*0.091	0.017	0.034	*0.042	*0.130
45	*0.078	0.007	0.015	*0.020	*0.086
#60	0.005	0.003	0.013	0.008	0.032
75	0.000	0.000	0.000	0.000	0.000
P-value	0.000	0.000	0.000	0.000	0.000

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	4.21	4.78	3.76	4.25	0.42
40	14.89	20.42	21.93	19.08	3.03
50	30.58	31.78	33.10	31.82	1.03
70	50.17	51.31	48.73	50.07	1.06
100	63.85	63.99	64.19	64.01	0.14

e. Scavenging activity of *M. selangorensis* KUM 20180 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2.7 *Marasmius* sp KUM 20067

a. Absorbance at 517 nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration stock extracts was

20 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.609	0.606	0.611	0.609	6.646	0.002
1	0.604	0.601	0.605	0.603	7.464	0.002
2	0.600	0.597	0.599	0.599	8.180	0.001
15	0.589	0.587	0.592	0.589	9.611	0.002
30	0.584	0.586	0.590	0.587	10.020	0.002
45	0.582	0.586	0.592	0.587	10.020	0.004
60	0.582	0.587	0.591	0.587	10.020	0.004
75	0.582	0.587	0.591	0.587	10.020	0.004

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.601	0.599	0.609	0.603	7.515	0.004
1	0.597	0.594	0.599	0.597	8.487	0.002
2	0.590	0.586	0.592	0.589	9.611	0.002
15	0.563	0.553	0.559	0.558	14.366	0.004
30	0.552	0.540	0.548	0.547	16.155	0.005
45	0.546	0.541	0.582	0.556	14.673	0.018
60	0.542	0.527	0.537	0.535	17.894	0.006
75	0.539	0.522	0.534	0.532	18.456	0.007

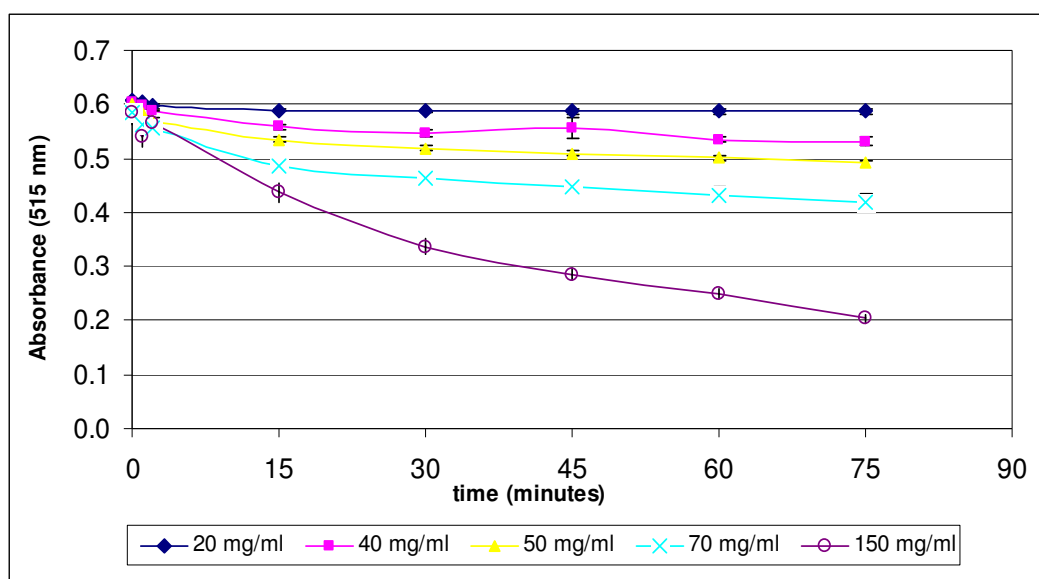
50 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.593	0.598	0.610	0.600	7.924	0.007
1	0.579	0.583	0.595	0.586	10.174	0.007
2	0.563	0.567	0.578	0.569	12.679	0.006
15	0.530	0.531	0.541	0.534	18.098	0.005
30	0.515	0.516	0.525	0.519	20.450	0.004
45	0.505	0.506	0.516	0.509	21.933	0.005
60	0.496	0.498	0.507	0.500	23.262	0.005
75	0.490	0.492	0.498	0.493	24.335	0.003

70 mg/ml						
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.572	0.583	0.599	0.585	10.327	0.011
1	0.563	0.557	0.572	0.564	13.497	0.006
2	0.555	0.562	0.550	0.556	14.775	0.005
15	0.491	0.472	0.498	0.487	25.307	0.011
30	0.467	0.446	0.476	0.463	28.988	0.013
45	0.451	0.430	0.462	0.448	31.339	0.013
60	0.437	0.414	0.447	0.433	33.640	0.014
75	0.424	0.401	0.435	0.420	35.583	0.014

150 mg/ml						
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.576	0.582	0.597	0.585	10.276	0.009
1	0.548	0.550	0.527	0.542	16.922	0.010
2	0.597	0.561	0.536	0.565	13.395	0.025
15	0.431	0.435	0.445	0.437	32.975	0.006
30	0.309	0.358	0.344	0.337	48.313	0.021
45	0.286	0.335	0.237	0.286	56.135	0.040
60	0.279	0.294	0.175	0.249	61.759	0.053
75	0.245	0.235	0.133	0.204	68.661	0.051

b. Graph of the mean of absorbance at 517 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius* sp **KUM 20067**: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	150mg/ml
0	*0.022	*0.068	*0.100	*0.152	*0.228
1	*0.017	*0.061	*0.085	*0.131	*0.185
2	*0.012	*0.054	*0.069	*0.123	*0.208
15	0.003	*0.023	*0.034	*0.054	*0.080
30	0.000	0.011	*0.018	*0.030	*0.044
45	0.000	*0.021	0.009	0.015	0.020
#60	0.000	0.004	0.007	0.013	0.020
75	0.000	0.000	0.000	0.000	0.000
P-value	0.000	0.000	0.000	0.000	0.000

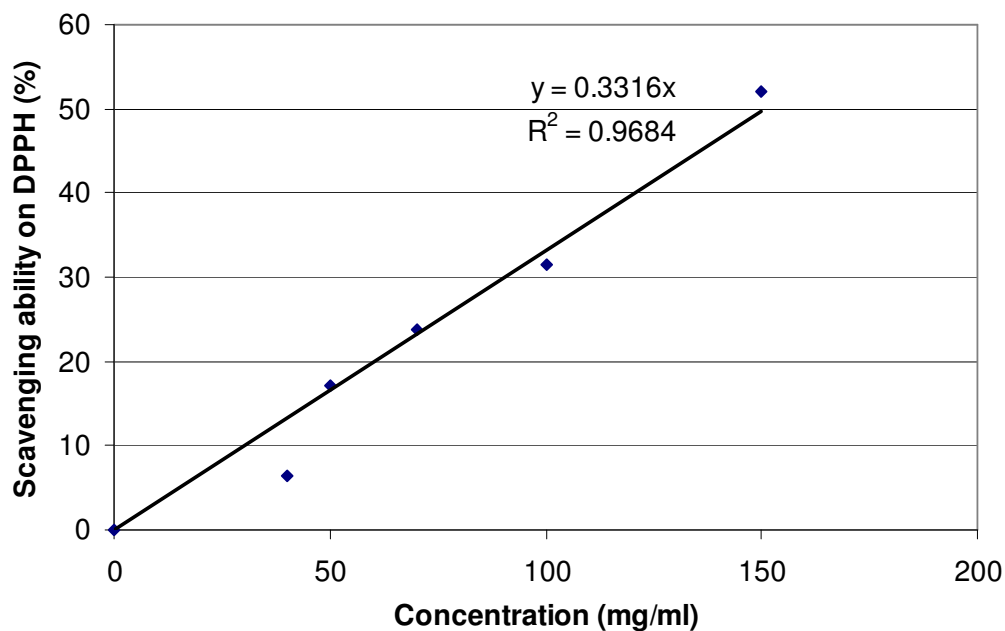
*Denotes the mean differences were significant ($P < 0.05$, ANOVA test)

Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH· radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	18.01	17.24	18.42	17.89	0.49
40	23.14	22.93	23.71	23.26	0.33
50	33.55	32.87	34.50	33.64	0.67
70	46.33	46.81	42.70	45.30	1.83
100	71.11	71.35	73.48	71.98	1.07

e. Scavenging activity of *Marasmius* sp. KUM 20067 on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2.8 *Marasmius kanchingnensis* KUM 20160

a. Absorbance at 517 nm in triplicates of the five different concentrations of stock extract and control, with calculated mean and standard deviation values for 0, 1, 2 and every 15 minutes interval. Concentration stock extracts was

20 mg/ml						
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.594	0.611	0.590	0.598	7.807	0.009
1	0.587	0.594	0.575	0.585	9.810	0.008
2	0.585	0.591	0.573	0.583	10.169	0.007
15	0.578	0.577	0.561	0.572	11.864	0.008
30	0.573	0.570	0.548	0.564	13.148	0.011
45	0.573	0.569	0.551	0.564	13.046	0.010
60	0.575	0.565	0.552	0.564	13.097	0.009
75	0.582	0.567	0.555	0.568	12.481	0.011

40 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.566	0.571	0.592	0.576	15.617	0.011
1	0.536	0.535	0.550	0.540	20.888	0.007
2	0.522	0.521	0.535	0.526	22.987	0.006
15	0.438	0.434	0.442	0.438	35.871	0.003
30	0.402	0.396	0.401	0.400	41.484	0.003
45	0.394	0.386	0.387	0.389	43.045	0.004
60	0.354	0.344	0.325	0.341	50.073	0.012
75	0.328	0.312	0.334	0.325	52.465	0.009

50 mg/ml

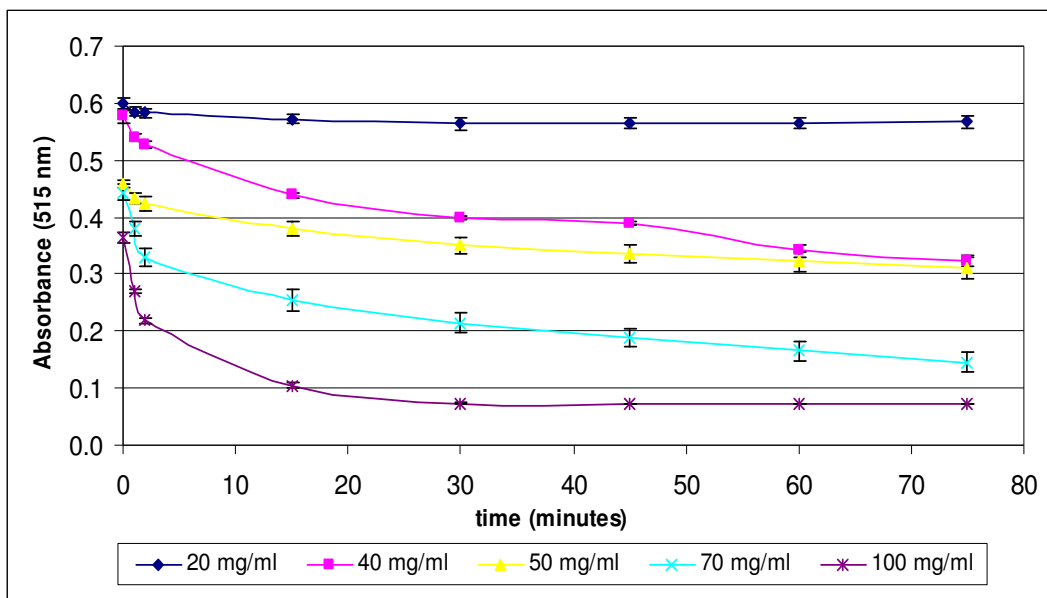
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.467	0.452	0.455	0.458	29.430	0.006
1	0.448	0.429	0.423	0.433	33.231	0.011
2	0.439	0.421	0.412	0.424	34.669	0.011
15	0.397	0.375	0.368	0.380	41.448	0.012
30	0.370	0.346	0.335	0.350	46.020	0.015
45	0.357	0.331	0.320	0.336	48.228	0.016
60	0.345	0.318	0.304	0.322	50.334	0.017
75	0.334	0.306	0.290	0.310	52.234	0.018

70 mg/ml

Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.452	0.423	0.455	0.443	31.690	0.014
1	0.396	0.372	0.368	0.379	41.654	0.012
2	0.352	0.317	0.319	0.329	49.255	0.016
15	0.280	0.235	0.250	0.255	60.709	0.019
30	0.237	0.197	0.208	0.214	67.026	0.017
45	0.211	0.171	0.182	0.188	71.032	0.017
60	0.189	0.148	0.160	0.166	74.474	0.017
75	0.168	0.127	0.140	0.145	77.658	0.017

100 mg/ml						
Time (minutes)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0	0.362	0.353	0.378	0.364	43.862	0.010
1	0.275	0.267	0.269	0.270	58.346	0.003
2	0.224	0.215	0.216	0.218	66.359	0.004
15	0.108	0.099	0.108	0.105	83.821	0.004
30	0.073	0.070	0.074	0.072	88.855	0.002
45	0.071	0.071	0.072	0.071	89.009	0.000
60	0.072	0.073	0.072	0.072	88.855	0.000
75	0.071	0.072	0.071	0.071	89.009	0.000

b. Graph of the mean of absorbance at 517 nm of the five different concentrations of extract at 0, 1, 2 and every 15 minutes interval.



*Each value is expressed as mean \pm standard deviation (n=3)

c. ANOVA test for *Marasmius ruforotula* KUM 20160: Analysis of variance for mean of absorbance values for every minute with the mean of absorbance values for at five different concentrations

Time interval	Means Differences				
	20 mg/ml	40 mg/ml	50 mg/ml	70 mg/ml	100mg/ml
0	*0.034	*0.235	*0.136	*0.278	*0.292
1	*0.021	*0.199	*0.111	*0.213	*0.198
2	0.019	*0.185	*0.102	*0.164	*0.146
15	0.008	*0.097	*0.058	*0.089	*0.033
30	0.0003	*0.059	*0.028	*0.048	0.000
45	0.0003	*0.048	0.014	0.022	0.001
#60	0.004	0.016	0.002	0.021	0.001

75	0.000	0.000	0.000	0.000	0.000
P-value	0.013	0.000	0.000	0.000	0.000

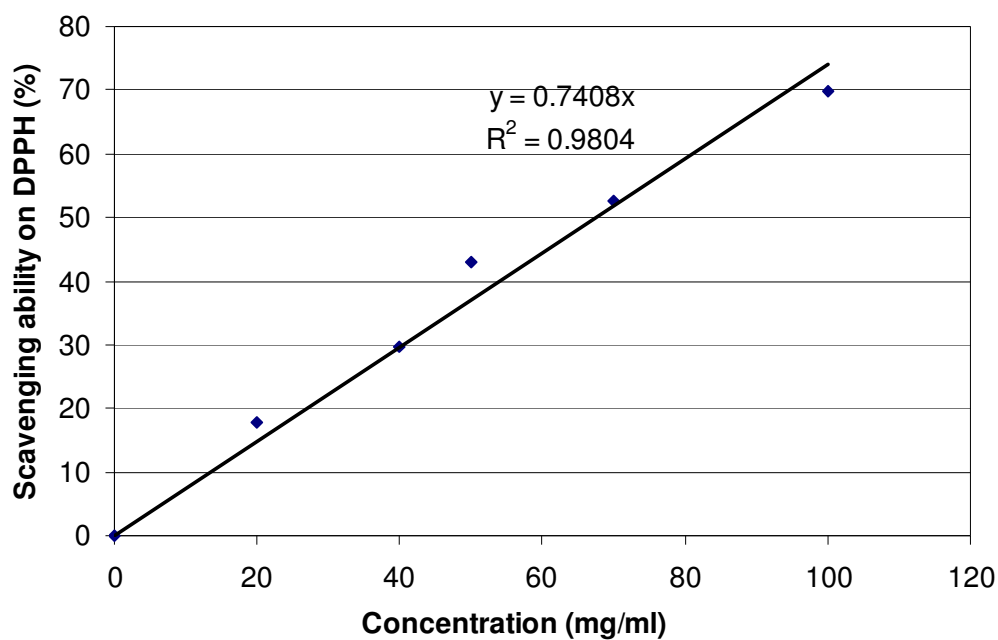
*Denotes the mean differences were significant ($P < 0.05$, ANOVA test)

#Denotes the mean values at 60 minutes for all the six concentrations of extracts did not show significant difference with the mean values at 75 minutes. Thus the extracts reached a steady state at 60 minutes

d. Percentage of DPPH radical scavenged by extracts at steady state (60 minutes) of the three replicates at five different concentrations with calculated mean and standard deviation.

Concentration of sample (mg/ml)	Percentage of inhibited DPPH radical				
	Test 1	Test 2	Test 3	Average	STDEV
20	13.02	13.07	13.21	13.10	0.08
40	49.44	47.38	47.87	48.23	0.88
50	50.40	50.60	49.99	50.33	0.25
70	75.27	74.15	73.99	74.47	0.57
100	87.39	89.99	89.17	88.85	1.09

e. Scavenging activity of *M. kanchingnensis* KUM 20160 on DPPH radicals. Each value is expressed as mean \pm standard deviation of triplicate measurements ($n = 3$).



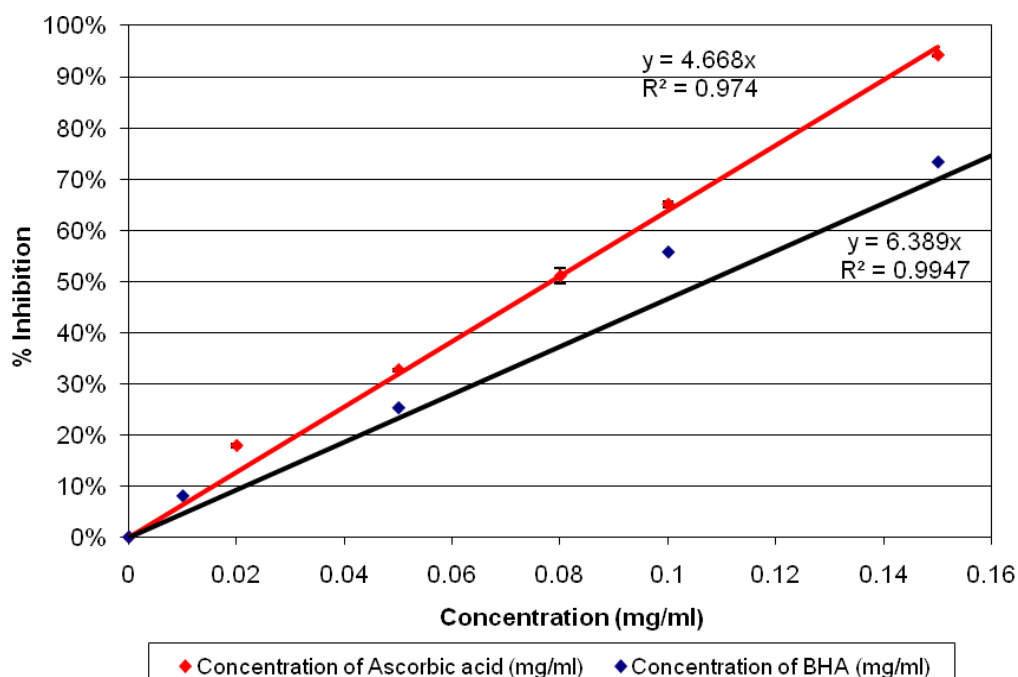
a. Absorbance at 517 nm in triplicates of the six different concentrations of BHA and control, with calculated mean and standard deviation values. Concentration stock extracts was mg/ml.

Concentration of BHA (mg/ml)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0.00	0	0	0	0	0	0
0.01	0.652	0.650	0.654	0.652	8.04%	0.002
0.05	0.498	0.577	0.515	0.530	25.25%	0.034
0.10	0.312	0.309	0.322	0.314	55.67%	0.006
0.15	0.200	0.206	0.163	0.190	73.25%	0.019
0.20	0.106	0.098	0.098	0.101	85.80%	0.004

a. Absorbance at 517 nm in triplicates of the six different concentrations of ascorbic acid and control, with calculated mean and standard deviation values. Concentration stock extracts was mg/ml.

Concentration of ascorbic acid (mg/ml)	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
0.00	0	0	0	0	0	0
0.02	0.503	0.508	0.499	0.503	17.89%	0.004
0.05	0.414	0.410	0.414	0.413	32.68%	0.002
0.08	0.284	0.319	0.312	0.305	51.04%	0.015
0.10	0.220	0.215	0.208	0.214	65.04%	0.005
0.15	0.036	0.037	0.034	0.036	94.18%	0.001

b. Scavenging activity of ascorbic acid and BHA on DPPH radicals. Each value is expressed as mean±standard deviation of triplicate measurements ($n = 3$).



2. Antioxidant activity of *Marasmius* spp. crude extracts using reducing power assay.

Reducing Power of butylated hydroxyanisole (BHA) (reference compound) at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	2.445	2.588	2.585	2.539	0.067
10	2.689	2.665	2.691	2.682	0.012
15	2.709	2.745	2.707	2.720	0.018
20	2.747	2.702	2.798	2.749	0.039

Reducing Power of (Ascorbic acid) (reference compound) at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	1.881	1.860	2.057	1.193	0.088
10	1.953	1.949	2.122	2.008	0.081
15	1.972	1.825	2.189	1.995	0.149
20	2.170	2.083	2.212	2.155	0.053

Reducing Power of Crude Dichloromethane Extracts from KUM 20111 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.265	0.278	0.294	0.279	0.012
10	0.389	0.364	0.394	0.382	0.013
15	0.543	0.652	0.529	0.575	0.055
20	0.902	0.834	0.877	0.877	0.028

Reducing Power of Crude Dichloromethane Extracts from KUM 20112 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.237	0.230	0.198	0.222	0.017
10	0.267	0.288	0.218	0.258	0.029
15	0.316	0.341	0.302	0.320	0.016
20	0.335	0.352	0.262	0.316	0.039

Reducing Power of Crude Dichloromethane Extracts from KUM 20022 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.016	0.062	0.133	0.070	0.048
10	0.107	0.114	0.127	0.116	0.008
15	0.096	0.199	0.108	0.134	0.046
20	0.154	0.149	0.167	0.157	0.008

Reducing Power of Crude Dichloromethane Extracts from KUM 20117 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.324	0.348	0.350	0.341	0.012
10	0.428	0.438	0.358	0.408	0.036
15	0.438	0.461	0.468	0.456	0.013
20	0.561	0.520	0.556	0.546	0.018

Reducing Power of Crude Dichloromethane Extracts from KUM 20044 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.562	0.532	0.395	0.496	0.073
10	0.742	0.793	0.769	0.768	0.021
15	0.996	0.983	0.972	0.984	0.010
20	1.130	1.178	1.124	1.144	0.024

Reducing Power of Crude Dichloromethane Extracts from KUM 20181 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.738	0.582	0.664	0.661	0.064
10	1.464	1.549	1.697	1.570	0.096
15	1.637	1.662	1.541	1.613	0.052
20	1.758	1.761	1.798	1.772	0.018

Reducing Power of Crude Dichloromethane Extracts from KUM 20160 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	1.079	1.086	1.072	1.079	0.006
10	1.321	1.260	1.300	1.294	0.025
15	1.397	1.529	1.304	1.410	0.092
20	1.495	1.583	1.479	1.519	0.046

Reducing Power of Crude Dichloromethane Extracts from KUM 20067 at Various Concentrations

Concentrations (mg/ml)	Reducing Power(absorbance reading at 700nm)				
	Test 1	Test 2	Test 3	Average	STDEV
5	0.550	0.510	0.230	0.430	0.142
10	0.993	0.992	0.995	0.993	0.001
15	1.300	1.280	1.310	1.297	0.012
20	1.450	1.400	1.310	1.387	0.058

3. Antioxidant activity of *Marasmius* spp. crude extracts using metal chelating assay.

Metal chelating of (EDTA) (reference compound) at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	0.038	0.037	0.040	0.038	95.68	0.001
2	0.033	0.030	0.031	0.031	96.47	0.001
3	0.019	0.020	0.014	0.018	98.01	0.003
4	0.012	0.010	0.019	0.014	98.46	0.004
5	0.007	0.013	0.011	0.010	98.84	0.002

Metal Chelating of Crude Dichloromethane Extracts from KUM 20111 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.084	0.977	1.024	1.028	11.91	0.044
2	0.972	0.964	1.053	0.996	14.65	0.040
3	0.951	0.968	0.969	0.963	17.53	0.008
4	0.927	0.948	0.947	0.941	19.42	0.010
5	0.868	0.866	0.858	0.864	25.99	0.004

Metal Chelating of Crude Dichloromethane Extracts from KUM 20112 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.137	1.032	1.01	1.060	9.22	0.055
2	1.04	0.938	0.93	0.984	15.68	0.045
3	1.04	0.963	0.926	0.976	16.36	0.047
4	0.916	0.961	1.01	0.962	17.56	0.038
5	0.854	0.962	0.919	0.912	21.90	0.044

Metal Chelating of Crude Dichloromethane Extracts from KUM 20022 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.080	0.969	1.046	1.032	12.521	0.046
2	0.958	0.946	0.926	0.943	20.011	0.013
3	0.894	0.934	0.927	0.918	22.131	0.017
4	0.861	0.851	0.862	0.858	27.247	0.005
5	0.800	0.801	0.803	0.801	32.052	0.001

Metal Chelating of Crude Dichloromethane Extracts from KUM 20117 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.149	1.132	1.134	1.138	4.92	1.149
2	1.043	1.033	1.210	1.095	8.52	1.043
3	1.030	0.937	1.128	1.032	13.84	1.030
4	0.857	0.965	0.934	0.919	23.27	0.857
5	0.865	0.939	0.859	0.888	25.86	0.865

Metal Chelating of Crude Dichloromethane Extracts from KUM 20044 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.160	1.151	1.120	1.144	3.24	0.017
2	1.130	1.117	1.149	1.132	4.23	0.013
3	1.160	0.980	1.148	1.096	7.28	0.082
4	0.950	0.961	0.978	0.963	18.53	0.012
5	0.940	0.812	0.951	0.901	23.77	0.063

Metal Chelating of Crude Dichloromethane Extracts from KUM 20181 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	0.654	0.659	0.655	0.656	25.85	0.002
2	0.614	0.623	0.624	0.620	29.88	0.004
3	0.606	0.611	0.608	0.608	31.24	0.002
4	0.576	0.585	0.594	0.585	33.87	0.007
5	0.572	0.563	0.565	0.567	35.95	0.004

Metal Chelating of Crude Dichloromethane Extracts from KUM 20160 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	0.854	0.850	1.043	0.916	21.56	0.090
2	0.894	0.79	0.809	0.831	28.81	0.045
3	0.802	0.788	0.79	0.793	32.04	0.006
4	0.765	0.789	0.776	0.777	33.47	0.010
5	0.767	0.769	0.775	0.770	34.01	0.003

Metal Chelating of Crude Dichloromethane Extracts from KUM 20067 at Various Concentrations

Concentrations (mg/ml)	Metal Chelating (absorbance reading at 700nm)					
	Test 1	Test 2	Test 3	Average	% inhibition	STDEV
1	1.190	1.087	1.081	1.119	5.09	1.190
2	1.144	0.937	1.127	1.069	10.29	1.144
3	1.017	0.976	0.987	0.993	15.77	1.017
4	1.046	0.938	0.852	0.945	19.84	1.046
5	0.834	0.854	0.795	0.828	29.82	0.834

4. Cytotoxic activity of eight *Marasmius* crude extracts against various cancer cell lines.

Percentage (%) of Growth Inhibition by Crude Dichloromethane Extracts of *Marasmius* spp. against CaSki Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test 1 (%)	Test 2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	20.45	22.11	19.33	20.63	1.14
<i>Marasmius ruforotula</i>	KUM 20112	20.39	25.71	22.55	22.89	2.19
<i>Marasmius guyanensis</i>	KUM 20022	17.14	16.82	12.20	15.39	2.26
<i>Marasmius</i> sp.	KUM 20067	35.65	30.65	29.78	32.03	2.59
<i>Marasmius selangorensis</i>	KUM 20181	7.77	10.43	12.88	10.36	2.09
<i>Marasmius kanchingnensis</i>	KUM 20160	14.81	13.26	17.05	15.04	1.55
<i>Marasmius guyanensis</i>	KUM 20117	18.40	16.82	18.70	17.97	0.82
<i>Marasmius guyanensis</i>	KUM 20044	8.08	7.77	10.48	8.77	1.21

Percentage (%) of Growth Inhibition by Crude Dichloromethane Extracts of *Marasmius* spp. against Skov 3 Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test1 (%)	Test2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	34.13	37.50	32.12	34.58	2.22
<i>Marasmius ruforotula</i>	KUM 20112	34.37	30.45	35.30	33.37	2.10
<i>Marasmius guyanensis</i>	KUM 20022	31.95	38.50	30.95	33.80	3.35
<i>Marasmius</i> sp.	KUM 20067	8.00	6.76	6.47	7.08	0.66
<i>Marasmius selangorensis</i>	KUM 20181	18.94	15.87	20.18	18.33	1.81
<i>Marasmius kanchingnensis</i>	KUM 20160	25.28	21.87	25.68	24.28	1.71
<i>Marasmius guyanensis</i>	KUM 20117	19.75	17.57	14.89	17.40	1.99
<i>Marasmius guyanensis</i>	KUM 20044	36.67	36.17	40.25	37.70	1.82

Percentage (%) of Growth Inhibition by Crude Dichloromethane Extracts of *Marasmius* spp. against HCT 119 Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test1 (%)	Test2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	37.20	32.35	41.46	37.00	3.72
<i>Marasmius ruforotula</i>	KUM 20112	36.05	30.84	39.56	35.48	3.58
<i>Marasmius guyanensis</i>	KUM 20022	4.57	2.73	4.47	3.93	0.84
<i>Marasmius</i> sp.	KUM 20067	19.34	10.92	13.63	14.63	3.51
<i>Marasmius selangorensis</i>	KUM 20181	36.32	28.21	35.61	33.38	3.67
<i>Marasmius kanchingnensis</i>	KUM 20160	9.35	3.64	7.76	6.92	2.40
<i>Marasmius guyanensis</i>	KUM 20117	31.42	28.39	28.27	29.36	1.46
<i>Marasmius guyanensis</i>	KUM 20044	17.01	16.93	18.27	17.40	0.61

Percentage (%) of Growth Inhibition by Crude Dichloromethane Extracts of *Marasmius* spp. against KB Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test1 (%)	Test 2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	48.07	46.51	46.13	46.90	0.84
<i>Marasmius ruforotula</i>	KUM 20112	47.84	44.40	49.26	47.17	2.04
<i>Marasmius guyanensis</i>	KUM 20022	10.32	14.49	12.42	12.41	1.70
<i>Marasmius</i> sp.	KUM 20067	27.46	24.49	23.60	25.18	1.65
<i>Marasmius selangorensis</i>	KUM 20181	48.56	48.34	43.79	46.90	2.20
<i>Marasmius kanchingnensis</i>	KUM 20160	17.42	18.37	13.92	16.57	1.91
<i>Marasmius guyanensis</i>	KUM 20117	36.62	39.80	33.45	36.62	2.59
<i>Marasmius guyanensis</i>	KUM 20044	6.83	7.70	12.32	8.95	2.41

Percentage (%) of Growth Inhibition by Crude dichloromethane Extracts of *Marasmius* spp. against HT 29 Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp. at 20		Percentage (%) of Growth Inhibition				
		Test 1 (%)	Test 2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	28.57	24.00	28.67	27.08	2.18
<i>Marasmius ruforotula</i>	KUM 20112	23.81	25.33	27.05	25.40	1.32
<i>Marasmius guyanensis</i>	KUM 20022	45.90	39.05	37.14	40.70	3.76
<i>Marasmius</i> sp.	KUM 20067	14.84	13.93	12.33	13.70	1.04
<i>Marasmius selangorensis</i>	KUM 20181	6.56	6.13	8.81	7.17	1.18
<i>Marasmius kanchingnensis</i>	KUM 20160	13.33	12.62	20.95	15.64	3.77
<i>Marasmius guyanensis</i>	KUM 20117	8.14	4.10	9.35	7.20	2.25
<i>Marasmius guyanensis</i>	KUM 20044	4.84	3.80	4.07	4.24	0.44

Percentage (%) of Growth Inhibition by Crude dichloromethane Extracts of *Marasmius* spp. against MCF 7 Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test1 (%)	Test 2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius ruforotula</i>	KUM 20111	19.95	14.42	17.85	17.41	2.28
<i>Marasmius ruforotula</i>	KUM 20112	14.16	18.96	16.12	16.41	1.97
<i>Marasmius guyanensis</i>	KUM 20022	22.21	25.05	22.39	23.21	1.30
<i>Marasmius</i> sp.	KUM 20067	9.72	7.16	6.64	7.84	1.35
<i>Marasmius selangorensis</i>	KUM 20181	5.27	7.57	6.87	6.57	0.96
<i>Marasmius kanchingnensis</i>	KUM 20160	21.62	17.67	24.70	21.33	2.88
<i>Marasmius guyanensis</i>	KUM 20117	8.17	9.57	10.38	9.37	0.91
<i>Marasmius guyanensis</i>	KUM 20044	6.43	10.17	6.63	7.74	1.72

Percentage (%) of Growth Inhibition by Crude dichloromethane Extracts of *Marasmius* spp. against MRC 5 Cell Line

Dichloromethane extracts of <i>Marasmius</i> spp.		Percentage (%) of Growth Inhibition				
		Test 1 (%)	Test 2 (%)	Test 3 (%)	Average (%)	STDEV
<i>Marasmius rufotula</i>	KUM 20111	7.92	7.43	7.96	7.77	0.24
<i>Marasmius rufotula</i>	KUM 20112	6.68	8.17	7.95	7.60	0.66
<i>Marasmius guyanensis</i>	KUM 20022	8.12	9.68	9.18	8.99	0.65
<i>Marasmius</i> sp.	KUM 20067	5.45	6.63	7.13	6.40	0.70
<i>Marasmius selangorensis</i>	KUM 20181	7.06	7.20	9.46	7.91	1.10
<i>Marasmius kanchingensis</i>	KUM 20160	2.97	2.81	2.25	2.68	0.31
<i>Marasmius guyanensis</i>	KUM 20117	6.82	7.64	5.12	6.53	1.05
<i>Marasmius guyanensis</i>	KUM 20044	4.45	5.10	3.64	4.40	0.60

Appendix C: Calculation of ANOVA test
ANOVA Table for Col_2 by Col_1

Analysis of Variance					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	805.645	7	115.092	15.70	0.0000
Within groups	117.256	16	7.3285		
Total (Corr.)	922.901	23			

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The ANOVA table decomposes the variance of Col_2 into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 15.7047, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean Col_2 from one level of Col_1 to another at the 95.0% confidence level. To determine which means are significantly different from which others, select Multiple Range Tests from the list of Tabular Option.

Multiple Range Tests for Col_2 by Col_1

Method: 95.0 percent LSD			
Col_1	Count	Mean	Homogeneous Groups
7	3	51.433	X
8	3	52.178	X
2	3	52.9027	X
6	3	53.4673	XX
5	3	57.6673	XX
1	3	59.4853	X
4	3	60.3917	X
3	3	69.9297	X

Contrast	Difference	+/- Limits
1 - 2	*6.58267	4.68575
1 - 3	*-10.4443	4.68575
1 - 4	-0.906333	4.68575
1 - 5	1.818	4.68575
1 - 6	*6.018	4.68575
1 - 7	*8.05233	4.68575
1 - 8	*7.30733	4.68575
2 - 3	*-17.027	4.68575
2 - 4	*-7.489	4.68575

2 - 5	*-4.76467	4.68575
2 - 6	-0.564667	4.68575
2 - 7	1.46967	4.68575
2 - 8	0.724667	4.68575
3 - 4	*9.538	4.68575
3 - 5	*12.2623	4.68575
3 - 6	*16.4623	4.68575
3 - 7	*18.4967	4.68575
3 - 8	*17.7517	4.68575
4 - 5	2.72433	4.68575
4 - 6	*6.92433	4.68575
4 - 7	*8.95867	4.68575
4 - 8	*8.21367	4.68575
5 - 6	4.2	4.68575
5 - 7	*6.23433	4.68575
5 - 8	*5.48933	4.68575
6 - 7	2.03433	4.68575
6 - 8	1.28933	4.68575
7 - 8	-0.745	4.68575

 * denotes a statistically significant difference.

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This table applies a multiple comparison procedure to determine which means are significantly different from which others. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 18 pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level. At the top of the page, 4 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.