Supporting information

Protective Effect of *Aframomum melegueta* phenolics Against CCl_4-Induced Rat Hepatocytes Damage; Role of Apoptosis and Pro-inflammatory Cytokines inhibition.

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Table 1

Effects of different extracts of *A. melegueta* and isolated compounds on alanine aminotransferase (ALT) activity induced by carbon tetrachloride using isolated suspended hepatocytes

ALT(U/L)					
	Time after addition of CCl_4 (min)				
Treatment	0	60	90	120	180
Normal (DMSO)	**39.29±2.02**	**41.26±2.41**	**40.67±1.62**	**38.08±2.11**	**41.29±1.72**
Control (CCl_4)	**42.55±2.18**	**94.03±3.26***	**95.22±6.62***	**98.33±4.26***	**119.24±6.88***
Curcumin (1 µM)	**38.32±2.33**	**42.82±2.81@**	**44.31±3.09@**	**48.63±2.81@**	**50.54±2.72@**
Methanol extract	**41.26±1.80**	**43.44±3.18@**	**48.48±4.88@**	**43.01±4.67@**	**49.12±3.11@**
Chloroform extract	**40.49±2.16**	**46.21±3.61@**	**49.23±4.19@**	**51.01±3.81@**	**45.81±3.61@**
Hexane extract	**48.17±2.84**	**51.39±2.83@**	**58.32±4.66@**	**58.67±4.22@**	**53.91±3.56@**
Ethylacetate extract	**39.18±2.96**	**78.47±2.05***	**81.06±3.55***	**79.78±3.82***	**73.67±3.98***
Butanol extract	**40.23±3.01**	**71.47±2.93***	**79.12±3.15***	**80.64±3.09***	**75.67±4.01***
1	**41.33±2.42**	**47.55±3.46@**	**49.77±3.82@**	**53.48±3.76@**	**46.31±3.83@**
2	**40.22±3.11**	**88.65±2.13***	**87.29±3.19***	**76.09±4.03***	**75.32±3.88***
3	**46.72±2.95**	**47.17±3.90@**	**49.66±4.01@**	**50.43±3.75@**	**48.59±3.72@**
4	**39.26±1.80**	**41.44±2.48@**	**48.48±4.88@**	**43.01±4.67@**	**49.12±3.11@**
5	**48.17±2.84**	**50.69±2.88@**	**53.52±4.29@**	**52.07±4.13@**	**52.01±3.70@**
6	**39.18±2.96**	**73.47±2.05***	**82.06±3.55***	**79.78±3.82***	**77.12±4.02***
7	**43.85±2.99**	**77.14±3.23***	**80.66±3.56***	**89.54±4.26***	**88.22±4.42***
8	**47.15±2.91**	**55.39±2.98@**	**54.32±5.01@**	**50.67±4.22@**	**51.19±3.01@**
9	**42.55±2.33**	**43.79±2.75@**	**49.17±3.90@**	**45.61±3.76@**	**49.95±3.84@**

Hepatocyte suspensions ($5x10^6$ cells/ml)in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2.The cells were incubated with the test compounds (dissolved in DMSO)for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 2

Effects of different extracts of *A. melegueta* and isolated compounds on glutathione (GSH) depletion induced by carbon tetrachloride using isolated suspended hepatocytes

	GSH(nmol/10^6 cells)				
	Time after addition of CCl_4 (min)				
Treatment	0	60	90	120	180
Normal (DMSO)	**61.45±5.05**	**59.07±4.15**	**55.67±4.45**	**54.55±4.23**	**53.49±4.59**
Control (CCl_4)	**62.42±4.93**	**22.12±2.90**	**19.67±2.85***	**17.96±1.05***	**13.09±0.90***
Curcumin (1 µM)	**60.25±4.78**	**54.13±2.24**	**49.67±3.02@**	**47.09±3.06@**	**43.91±3.89@**
Methanol extract	**61.08±4.64**	**53.55±3.38**	**50.32±3.68@**	**48.42±3.41@**	**46.02±3.88@**
Chloroform extract	**59.67±4.02**	**49.99±3.98**	**43.19±3.27@**	**41.46±3.24@i**	**37.29±3.22@**
Hexane extract	**60.23±4.35**	**48.67±3.06**	**40.67±3.45@**	**39.28±2.69*@**	**36.71±1.89*@**
Ethyl acetate extract	**60.37±2.22**	**27.67±2.11***	**29.67±2.45***	**25.40±1.70***	**23.94±1.93***
Butanol extract	**58.24±2.47**	**23.67±2.91***	**24.67±1.74***	**21.40±1.48***	**19.94±1.88***
1	**59.11±3.86**	**48.52±3.78**	**50.29±3.44@**	**51.33±3.16@**	**51.29±3.41@**
2	**58.19±3.45**	**27.53±2.54***	**29.71±2.32***	**28.37±1.82***	**25.71±1.98***
3	**58.67±2.29**	**23.51±2.79***	**24.72±1.74***	**21.16±1.44***	**19.94±1.58***
4	**61.11±4.34**	**44.13±2.64**	**48.52±2.15@**	**50.36±3.44@**	**49.91±3.89@**
5	**57.81±4.66**	**48.67±3.12**	**47.22±3.18@**	**49.75±2.42*@**	**46.71±2.02*@**
6	**58.43±2.61**	**28.42±2.61***	**30.67±2.68***	**32.40±1.66***	**31.48±1.93***
7	**57.44±2.57**	**26.32±3.01***	**21.43±1.82***	**20.62±1.73***	**20.73±1.91***
8	**60.67±4.02**	**50.99±3.32**	**41.19±3.54@**	**40.46±3.84@**	**45.29±3.66@**
9	**57.27±4.59**	**49.18±3.22**	**46.81±3.58@**	**42.28±2.55*@**	**41.17±2.47*@**

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2. The cells were incubated with the different extracts (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 3

Effects of different extracts of *A. melegueta* and isolated compounds on the formation of thiobarbituric acid reactive substances (TBARS) induced by carbon tetrachloride using isolated suspended hepatocytes

	TBARS (nmol/10^6 cells)				
	Time after addition of CCl_4 (min)				
Treatment	0	60	90	120	180
Normal (DMSO)	**0.036±0.006**	**0.043±0.005**	**0.039±0.005**	**0.040±0.004**	**0.042±0.006**
Control (CCl_4)	**0.038±0.005**	**0.197±0.032***	**0.287±0.040***	**0.284±0.047***	**0.301±0.058***
Curcumin (1µM)	**0.036±0.003**	**0.124±0.024@**	**0.189±0.051*@**	**0.205±0.053*@**	**0.183±0.008*@**
Methanol extract	**0.036±0.004**	**0.063±0.013@**	**0.107±0.026*@**	**0.099±0.032*@**	**0.086±0.009*@**
Chloroform extract	**0.042±0.008**	**0.085±0.015***	**0.093±0.025***	**0.090±0.031*@**	**0.096±0.016*@**
Hexane extract	**0.041±0.006**	**0.110±0.028*@**	**0.132±0.044*@**	**0.134±0.060*@**	**0.119±0.012*@**
Ethyl acetate extract	**0.036±0.003**	**0.124±0.024***	**0.189±0.051***	**0.225±0.053***	**0.283±0.008***
Butanol extract	**0.037±0.002**	**0.214±0.039***	**0.229±0.048***	**0.246±0.061***	**0.267±0.0063***
1	**0.038±0.006**	**0.102±0.032*@**	**0.141±0.051*@**	**0.121±0.059*@**	**0.099±0.014*@**
2	**0.042±0.008**	**0.155±0.020***	**0.223±0.068***	**0.290±0.081***	**0.288±0.069***
3	**0.037±0.002**	**0.204±0.043***	**0.234±0.048***	**0.233±0.061***	**0.269±0.063***
4	**0.036±0.004**	**0.165±0.013***	**0.217±0.026***	**0.263±0.071***	**0.286±0.078***
5	**0.041±0.007**	**0.110±0.028*@**	**0.132±0.044*@**	**0.134±0.060*@**	**0.119±0.012*@**
6	**0.036±0.003**	**0.224±0.024***	**0.247±0.051***	**0.259±0.053***	**0.291±0.081***
7	**0.041±0.006**	**0.156±0.052***	**0.198±0.067***	**0.224±0.089***	**0.232±0.057***
8	**0.038±0.004**	**0.075±0.016@**	**0.115±0.028*@**	**0.097±0.029*@**	**0.074±0.010*@**
9	**0.036±0.003**	**0.069±0.015@**	**0.121±0.022*@**	**0.082±0.032*@**	**0.066±0.011*@**

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2. The cells were incubated with the different extracts (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 4

Effects of different extracts of *A. melegueta* and isolated compounds on the levels of tumor necrosis factor alpha (TNF-α) induced by carbon tetrachloride using isolated suspended hepatocytes

	TNF-α (pg/ml)				
	Time after addition of CCl_4 (min)				
Treatment	**0**	**60**	**90**	**120**	**180**
Normal (DMSO)	**102.25±9.32**	**101.45±6.16**	**98.12±6.77**	**96.18±7.56**	**92.25±3.56**
Control(CCl_4)	**99.34±6.61**	**230.75±10.12***	**245.75±11.01***	**261.75±13.98***	**251.75±11.54***
Curcumin (1 µM)	**98.75±7.16**	**129.02±6.35@**	**137.02±9.44*@**	**148.02±1.28*@**	**158.02±7.95*@**
Methanol extract	**98.75±7.16**	**129.02±6.35***	**117.02±9.44*@**	**128.02±5.28*@**	**141.02±7.95*@**
Chloroform extract	**96.11±7.55**	**132.34±9.32@**	**128.24±9.08@**	**146.34±1.28@**	**155.34±9.84@**
Hexane extract	**103.17±5.98**	**145.02±7.55***	**139.02±8.37*@**	**146.02±1.28*@**	**154.02±9.28*@**
Ethyl acetate extract	**101.22±8.46**	**217.80±8.23***	**212.40±10.82***	**214.80±12.43***	**220.80±10.22***
Butanol extract	**99.55±6.14**	**201.02±6.15***	**189.02±7.24***	**210.02±9.28***	**233.06±8.05***
1	**103.17±5.98**	**145.02±7.55***	**139.02±8.37*@**	**146.02±1.28*@**	**154.02±9.28*@**
2	**103.56±6.82**	**237.11±9.81***	**200.55±11.01***	**216.80±12.27***	**232.75±11.18***
3	**101.22±8.46**	**217.80±8.23***	**212.40±10.82***	**214.80±12.43***	**220.80±10.22***
4	**99.34±6.61**	**230.35±10.12***	**245.61±11.01***	**261.95±13.98***	**251.14±13.54***
5	**98.75±7.16**	**129.87±6.35***	**117.38±9.44*@**	**128.25±5.28*@**	**141.33±7.95*@**
6	**99.55±6.14**	**201.02±6.15***	**189.02±7.24***	**210.02±9.28***	**233.06±8.05***
7	**99.34±6.61**	**196.66±10.41***	**206.37±12.07***	**223.49±13.32***	**225.66±13.11***
8	**100.02±6.46**	**116.58±6.13***	**124.72±8.19*@**	**130.66±5.15*@**	**138.21±8.03*@**
9	**96.11±7.55**	**132.34±9.32@**	**128.24±9.08@**	**146.34±1.28@**	**155.34±9.84@**

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2. The cells were incubated with the different extracts (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 5

Effects of different extracts of *A. melegueta* and isolated compounds on the levels of interleukin 1-beta (IL-1β) induced by carbon tetrachloride using isolated suspended hepatocytes

IL-1β (pg/ml)					
	Time after addition of CCl_4(min)				
Treatment	**0**	**60**	**90**	**120**	**180**
Normal(DMSO)	**34.12±1.69**	**34.15±1.53**	**51.14±1.72**	**43.44±9.42**	**42.22±2.42**
Control(CCl_4)	**35.22±2.56**	**205.34±8.56***	**191.34±6.56***	**159.34±7.88***	**167.54±9.17***
Curcumin (1 µM)	**32.66±2.87**	**96.88±5.09*@**	**95.28±4.01*@**	**74.33±7.51@**	**61.54±4.02@**
Methanol extract	**35.18±1.98**	**82.23±4.01*@**	**90.68±4.14*@**	**81.23±5.13@**	**48.91±2.42@**
Chloroform extract	**32.66±2.87**	**96.88±5.09*@**	**95.28±4.01*@**	**74.33±7.51@**	**61.54±9.06@**
Hexane extract	**35.23±2.56**	**86.28±4.77*@**	**97.19±4.58*@**	**80.37±6.16@**	**67.91±4.02@**
Ethyl acetate extract	**35.78±2.63**	**191.12±9.01***	**150.68±5.54***	**125.16±8.43***	**119.21±7.51***
Butanol extract	**34.94±2.42**	**188.59±5.87***	**178.48±5.24***	**132.46±5.51***	**118.38±3.92***
1	**35.17±3.01**	**91.54±5.27*@**	**90.28±4.58*@**	**77.65±7.53@**	**60.89±4.11@**
2	**37.67±2.65**	**181.48±9.68***	**167.12±5.79***	**148.19±8.64***	**139.62±8.04***
3	**35.78±2.63**	**178.65±9.32***	**145.68±6.55***	**139.16±8.03***	**131.52±6.63***
4	**38.33±2.68**	**171.75±5.88***	**180.16±5.85***	**170.18±6.77***	**159.44±6.02***
5	**35.55±2.85**	**87.39±4.86*@**	**97.17±4.47*@**	**82.30±6.43@**	**68.91±4.49@**
6	**35.16±1.98**	**186.73±4.26***	**193.16±4.65***	**161.23±5.13***	**148.91±3.82***
7	**34.55±2.87**	**168.12±5.63***	**156.48±5.19***	**142.88±6.11***	**128.33±3.89***
8	**32.18±2.77**	**86.33±5.54*@**	**97.45±4.60*@**	**84.65±7.57@**	**71.14±4.54@**
9	**33.98±3.06**	**81.22±4.34*@**	**90.57±4.58*@**	**80.37±6.88@**	**77.67±4.51@**

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2. The cells were incubated with the test compound (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 6

Effects of different compounds on caspase-3 activity induced by carbon tetrachloride using isolated suspended hepatocytes

Caspase-3 activity(U/well)					
	Time after addition of CCl_4 (min)				
Treatment	**0**	**60**	**90**	**120**	**180**
Normal (DMSO)	**0.65±0.049**	**0.63±0.032**	**0.69±0.045**	**0.68±0.053**	**0.72±0.036**
Control (CCl_4)	**1.37±0.065***	**1.39±0.051***	**1.51±0.049***	**1.53±0.058***	**1.68±0.061***
Curcumin (1 µM)	**0. 63±0.039@**	**0.66±0.042@**	**0.69±0.048@**	**0.75±0.072@**	**0.81±0.069@**
1	**0.68±0.053@**	**0.72±0.042@**	**0.74±0.071@**	**0.81±0.059@**	**0.89±0.044@**
2	**1.32±0.059***	**1.35±0.070***	**1.43±0.068***	**1.49±0.071***	**1.58±0.079***
3	**1.37±0.042**	**1.54±0.043***	**1.64±0.068***	**1.73±0.051***	**1.79±0.049***
4	**1.25±0.089***	**1.26±0.063***	**1.37±0.086***	**1.43±0.091***	**1.56±0.072***
5	**0.60±0.027@**	**0.69±0.058@**	**0.72±0.084@**	**0.84±0.068@**	**0. 91±0.012@**
6	**1.36±0.033**	**1.44±0.054***	**1.47±0.051***	**1.59±0.063***	**1.91±0.081***
7	**1.41±0.063***	**1.46±0.052***	**1.48±0.077***	**1.54±0.069***	**1.58±0.067***
8	**0. 59±0.049@**	**0.61±0.036@**	**0.65±0.028@**	**0.77±0.059@**	**0.84±0.070@**
9	**0.71±0.003@**	**0. 75±0.015@**	**0.81±0.022@**	**0.82±0.032*@**	**0.86±0.011@**

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2 . The cells were incubated with three concentrations of the test compound curcumin (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

@Significantly different from control group $p<0.05$.

Table 7

Effects of different compounds on caspase-9 activity induced by carbon tetrachloride using isolated suspended hepatocytes

Caspase-9 activity(U/well)

Time after addition of CCl_4 (min)

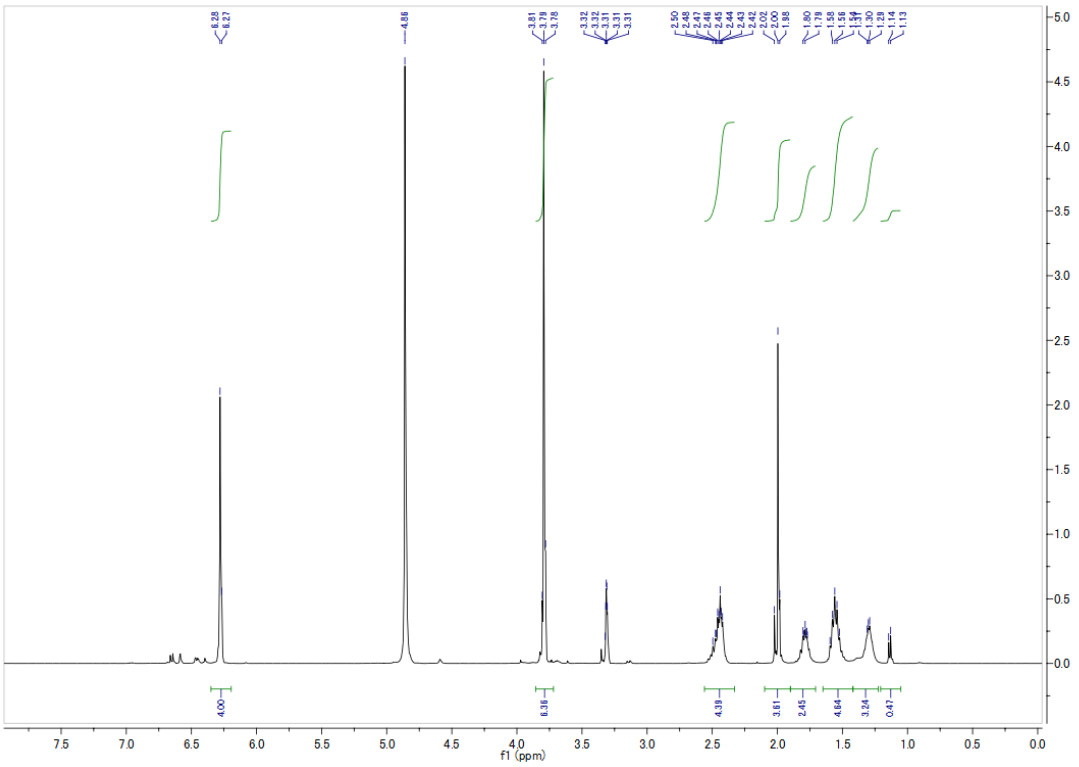
Treatment	0	60	90	120	180
Normal (DMSO)	0.49±0.059	0.51±0.054	0.55±0.062	0.60±0.53	0.59±0.061
Control (CCl_4)	1.02±0.032*	1.22±0.031*	1.41±0.039*	1.49±0.048*	1.68±0.061*
Curcumin (1 µM)	0.56±0.072@	0.60±0.054@	0.63±0.055@	0.68±0.071@	0.70±0.081@
1	0.63±0.045@	0.62±0.042@	0.54±0.061@	0.61±0.069@	0.69±0.074@
2	1.15±0.030*	1.15±0.033*	1.48±0.058*	1.39±0.071*	1.28±0.056*
3	1.19±0.042	1.24±0.073*	1.34±0.038*	1.43±0.061*	1.69±0.068*
4	1.17±0.041*	1.19±0.023*	1.21±0.022*	1.23±0.031*	1.29±0.028*
5	0. 41±0.055@	0.40±0.032@	0.52±0.053@	0.64±0.040@	0.69±0.072@
6	1.03±0.027	1.24±0.034*	1.27±0.051*	1.59±0.083*	1.58±0.092*
7	1.15±0.042*	1.16±0.082*	1.19±0.044*	1.24±0.091*	1.32±0.075*
8	0.38±0.014@	0.65±0.036@	0.62±0.048@	0.67±0.069@	0.74±0.060@
9	0.36±0.013@	0.49±0.025@	0.51±0.052@	0.62±0.082@	0.66±0.071@

Hepatocyte suspensions ($5x10^6$ cells/ml) in Krebs-Henseleit buffer were maintained at 37°C under an atmosphere of 95% O_2 and 5% CO_2 . The cells were incubated with three concentrations of the test compound curcumin (dissolved in DMSO) for 30 min. prior to the addition of the hepatotoxic CCL_4. Carbon tetrachloride was added to the incubation media in all groups at a concentration of 5 mM, except in the normal group where only the vehicle DMSO was added.

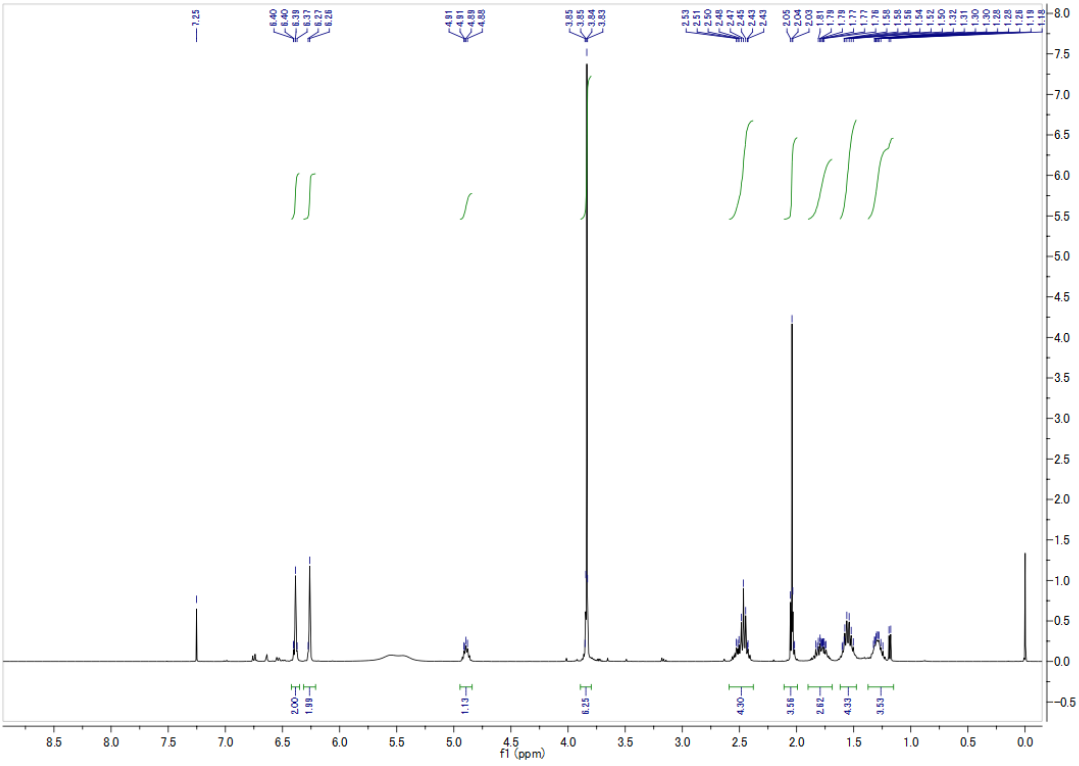
Each value represents the mean of six separate determinations ± SE of the mean.

* Significantly different from the normal group $p<0.05$.

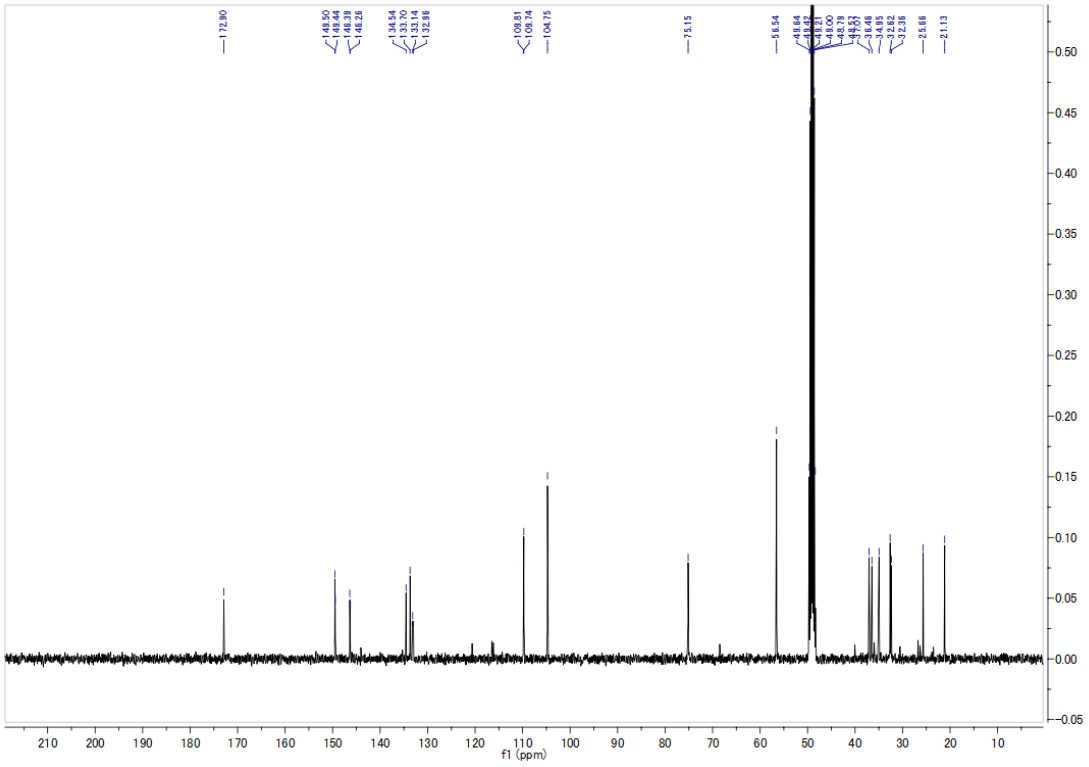
@Significantly different from control group $p<0.05$.



^{1}H-NMR chart of compound 1(CD3OD)



^{1}H-NMR of compound 1 (CDCl3)



13C-NMR of compound 1 (CD3OD)

HREIMS of compound 1

Sample: 9-5-8
Instrument: JEOL GCmate
Inlet: Direct Probe

Ionization mode: EI+

Scan: 20
Base: m/z 434; 100%FS TIC: 50630352

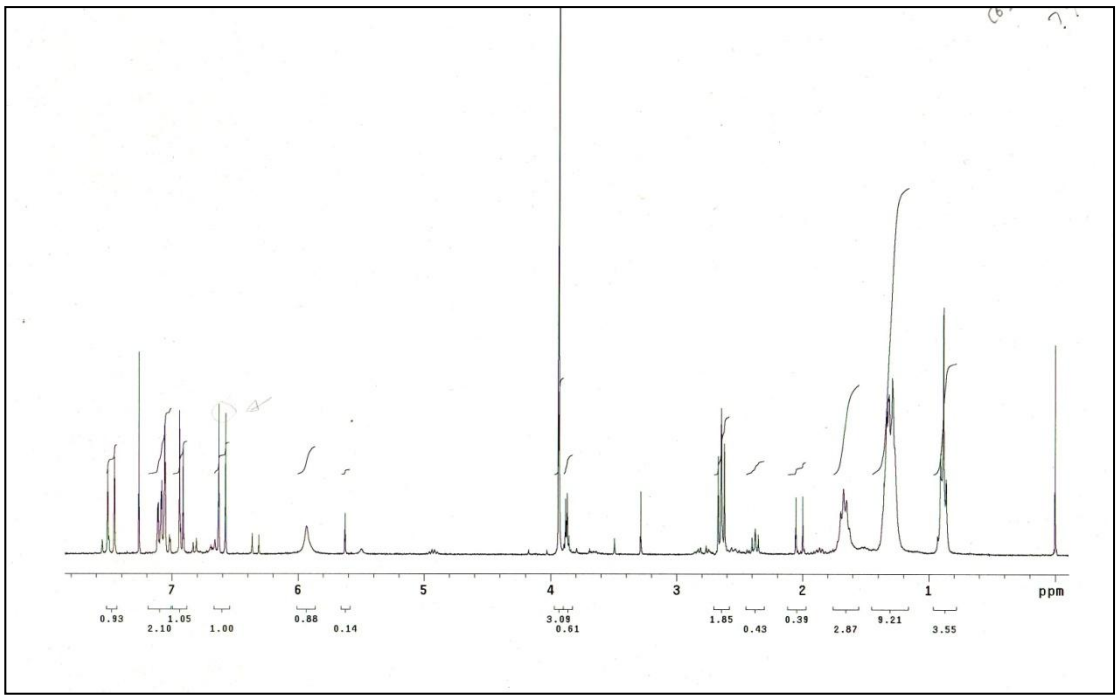
R.T.: 3.23

#Ions: 1743

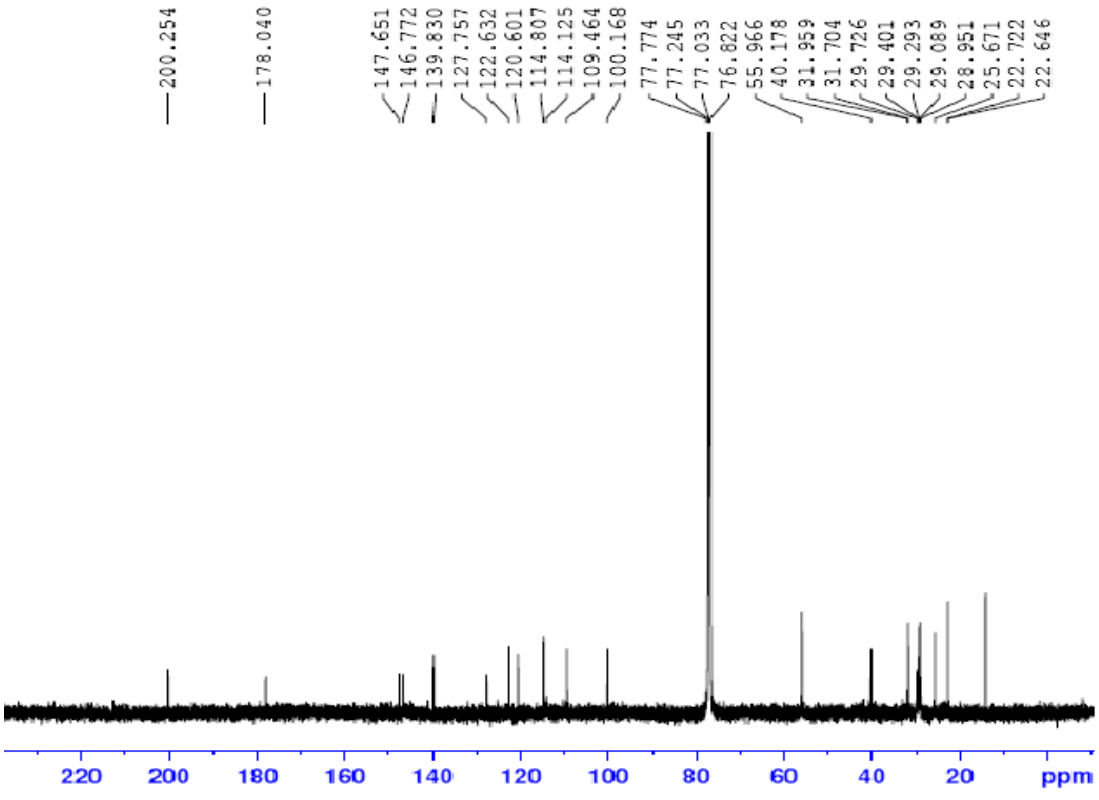
Selected Isotopes : H $C_{0-23}O_{0-8}$

Error Limit : 10 mmu

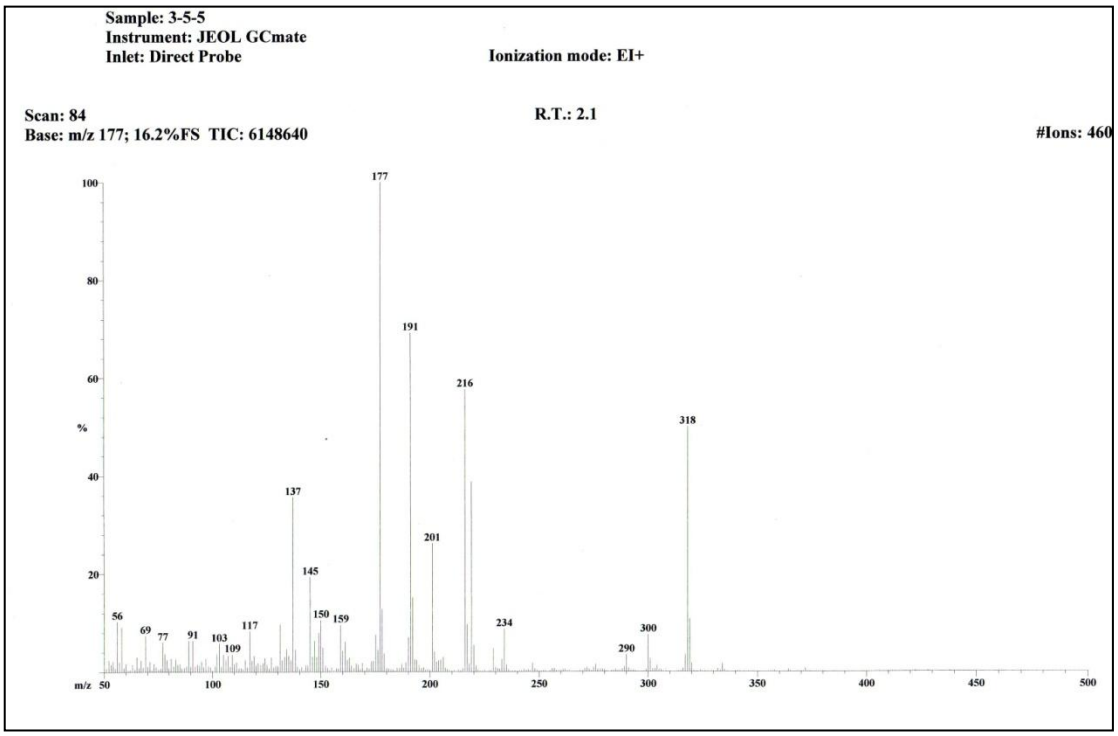
Measured Mass	*% Base*	*Formula*	*Calculated Mass*	*Error*
434.1874	100.0%	$C_{23}H_{30}O_8$	434.1941	-6.7



^{1}H-NMR chart of compound 2



^{13}C-NMR chart of compound 2



EI-MS of compound 2

Sample: 3-5-5)
Instrument: JEOL GCmate
Inlet: Direct Probe

Ionization mode: EI+

Scan: 13
Base: m/z 177; 100%FS TIC: 39500032

R.T.: 2.05

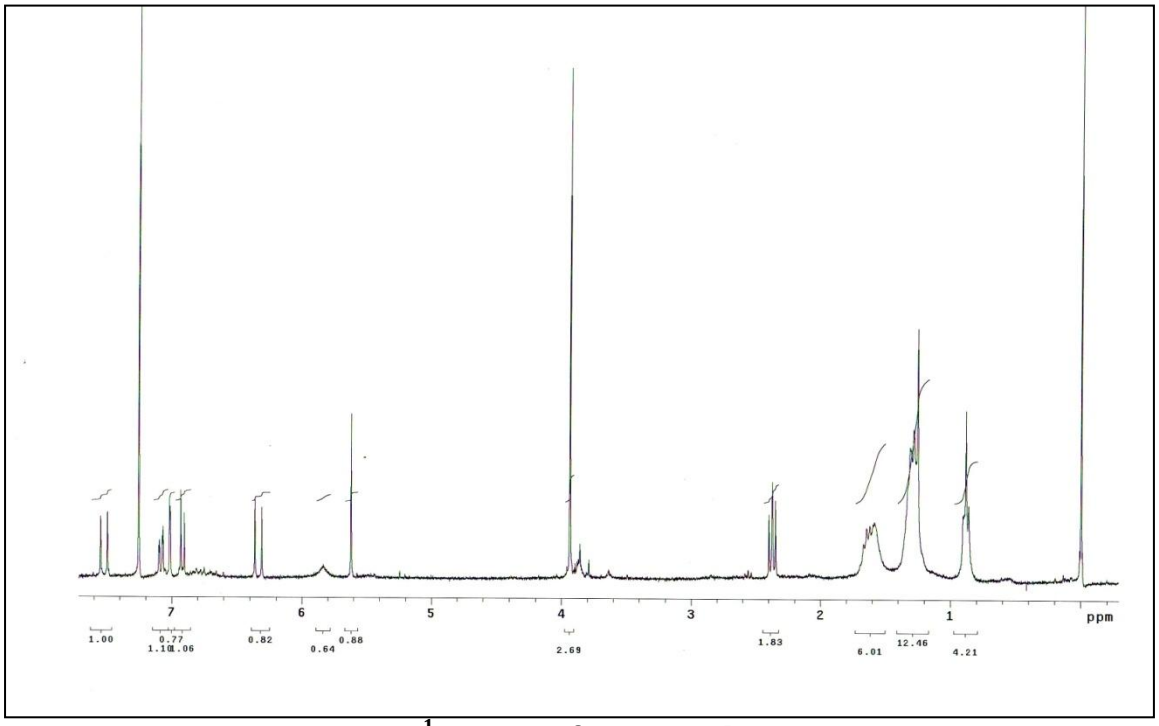
#Ions: 1029

Selected Isotopes : H $C_{0-19}O_{0-4}$

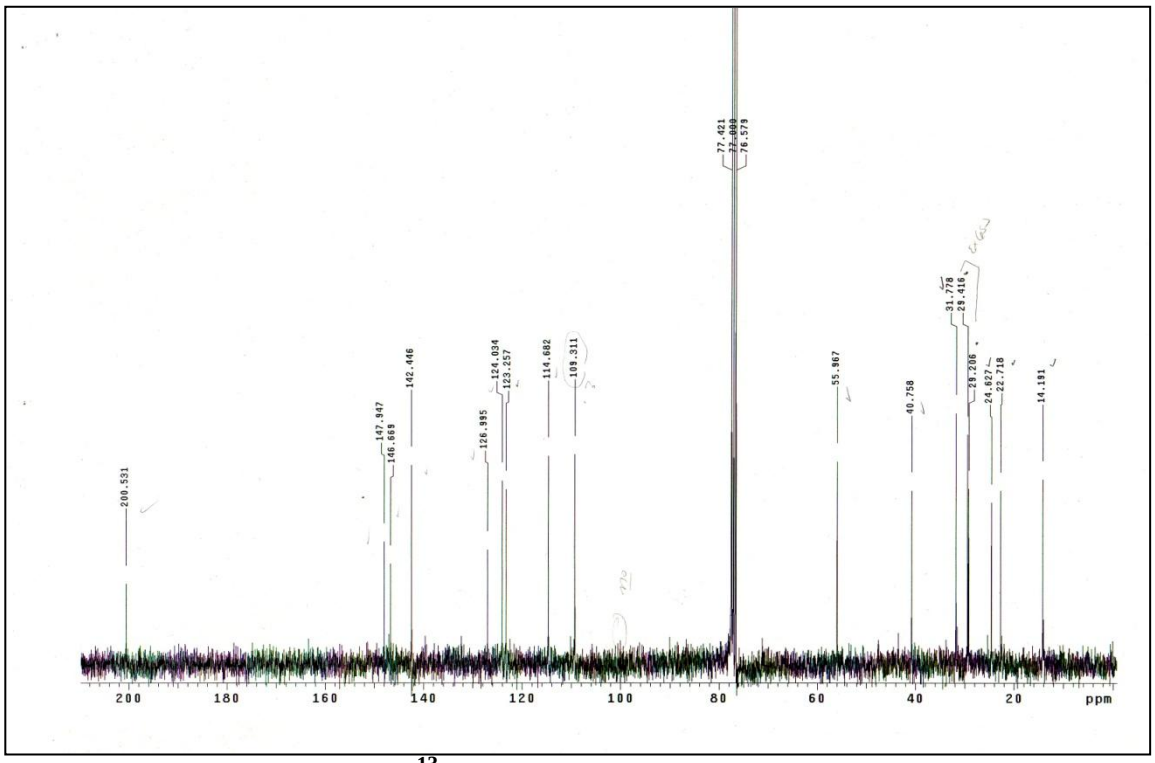
Error Limit : 10 mmu

Measured Mass	*% Base*	*Formula*	*Calculated Mass*	*Error*
318.1800	41.7%	$C_{19}H_{26}O_4$	318.1831	-3.1

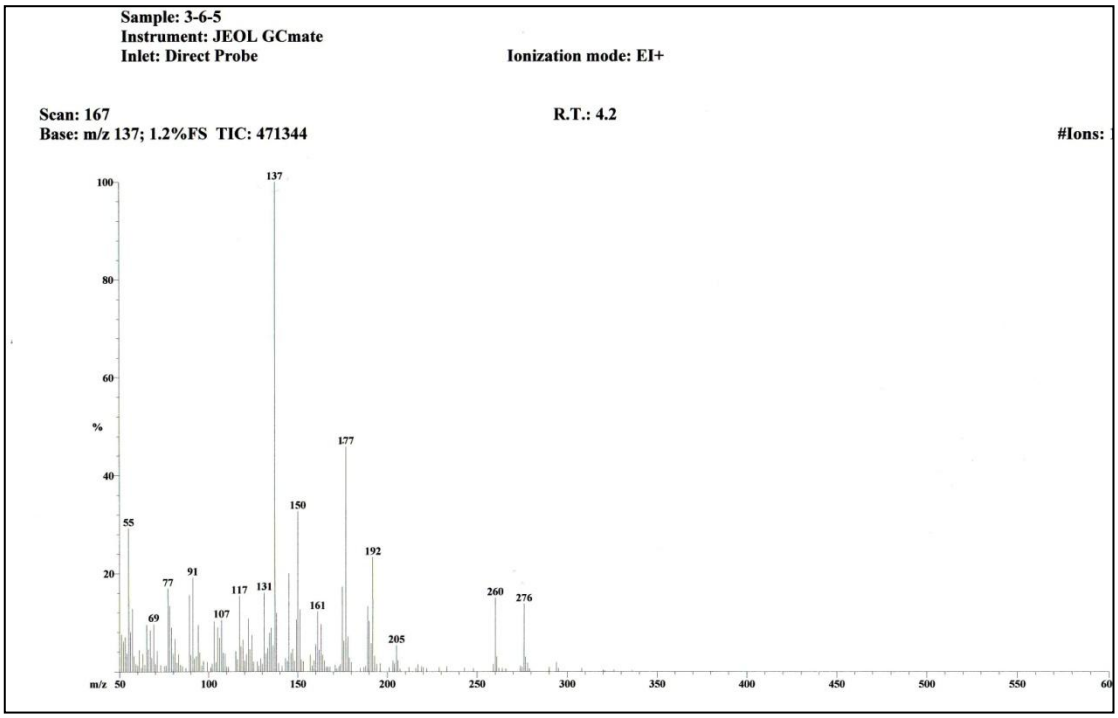
HREIMS of compound 2



^{1}H-NMR of compound 3



^{13}C-NMR of compound 3



EI-MS of compound 3