

APPENDIX 1

STATISTIC ANALYSIS

1. Stability of Limonin from Lime Seeds in Aqueous Solution

Table 1A Test of effects of temperature, pH, and time of experiments and between the subjects on remaining concentration of limonin in aqueous solution by all-3 ways UNIANOVA

Dependent Variable: remaining concentration of limonin in aqueous solution

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	49935.673a	279	178.981	4697.15	.000
Intercept	78766.460	1	78766.460	2067135.09	.000
TEMP	587.966	2	293.983	7715.24	.000
pH	33651.404	7	4807.343	126163.20	.000
TIME	27475.969	15	1831.731	48071.68	.000
TEMP * pH	310.635	14	22.188	582.30	.000
TEMP * TIME	223.590	28	7.985	209.57	.000
pH * TIME	3552.610	77	46.138	1210.83	.000
TEMP * pH * TIME	90.702	136	.667	17.50	.000
Error	21.338	560	3.810E-02		
Total	222972.668	840			
Corrected Total	49957.012	839			

a R Squared = 1.000 (Adjusted R Squared = .999)

Table 2A Homogenous subset of temperature effect on remaining concentration of limonin in aqueous solution by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test

TEMPERATURE	N	Subset		
		1	2	3
70	294	13.8268		
80	246		14.1291	
45	300			15.0486
Sig		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square (Error) = 3.810E-02.

1 Uses Harmonic Mean Sample Size = 277.785.

2 The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

3 Alpha = .05.

Table 3A Homogenous subset of pH effect on remaining concentration of limonin in aqueous solution by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test

pH	N	Subset							
		1	2	3	4	5	6	7	8
9	66	8.5923							
8	90		8.8952						
3	93			9.0951					
2	93				9.2333				
4	102					15.0568			
6	129						17.0291		
7	129							19.0867	
5	138								20.2064
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed, based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.810×10^{-02} .

1 Uses Harmonic Mean Sample Size = 99.599.

2 The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

3 Alpha = .05.

2. Stability of Limonin from Lime Seeds in Solid State

Table 4A Test of effects of temperature, days of storage and between the subjects on remaining concentration of limonin in solid state by all-2-ways UNIANOVA

Dependent Variable: percentage of remaining concentration of limonin

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35598.051a	41	868.245	5327.046	.000
Intercept	668981.580	1	668981.580	4104481.246	.000
TEMP	3572.796	2	1786.398	10960.297	.000
DAY	31812.385	13	2447.107	15014.020	.000
DAY * TEMP	212.870	26	8.187	50.233	.000
Error	13.691	84	.163		
Total	704593.322	126			
Corrected Total	35611.742	125			

a R Squared = 1.000 (Adjusted R Squared = .999)

Table 5A Homogenous subset of temperature effect on remaining concentration of limonin in solid state by UNINANOVA with Scheffe's Multiple Contrasts Post Hoc test

TEMPERATURE	N	Subset		
		1	2	3
70	42	66.1969		
80	42		73.1698	
45	42			79.2298
Sig		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares
The error term is Mean Square(Error) = .163.

1 Uses Harmonic Mean Sample Size = 42.000.

2 Alpha = .05.

3. Immunomodulation Effect of Limonin from Lime Seeds on Peritoneal Exudated Cells

Table 6A The tests of effects of groups of mice fed with different concentration of limonin, days of administration and between the subjects on percentage of phagocytosis (PP), phagocytosis index (PI) and peritoneal exudate cell number (PEC) by MANOVA

Dependent Variable: PEC number, PP, and PI							
Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	PP	87957.075a	23	3824.221	1763.932	.000	
	PI	13.974b	23	.608	2187.835	.000	
	PEC	4833428.906c	23	210149.083	533.830	.000	
Intercept	PP	345888.060	1	345888.060	159541.799	.000	
	PI	58.920	1	58.920	212172.212	.000	
	PEC	14589902.344	1	14589902.344	37061.891	.000	
GROUP	PP	87104.233	7	12443.462	5739.580	.000	
	PI	13.690	7	1.956	7042.432	.000	
	PEC	4718683.073	7	674097.582	1712.371	.000	
DAY	PP	479.158	2	239.579	110.506	.000	
	PI	.147	2	7.348E-02	264.597	.000	
	PEC	75282.812	2	37641.406	95.618	.000	
GROUP * DAY	PP	373.685	14	26.692	12.312	.000	
	PI	.137	14	9.799E-03	35.285	.000	
	PEC	39463.021	14	2818.787	7.160	.000	
Error	PP	156.097	72	2.168			
	PI	1.999E-02	72	2.777E-04			
	PEC	28343.750	72	393.663			
Total	PP	434001.232	96				
	PI	72.913	96				
	PEC	19451675.000	96				
Corrected Total	PP	88113.172	95				
Total	PI	13.994	95				
	PEC	4861772.656	95				

a R Squared = .998 (Adjusted R Squared = .998)

b R Squared = .999 (Adjusted R Squared = .998)

c R Squared = .994 (Adjusted R Squared = .992)

Table 7A Homogenous subset of group of mice fed with different concentration of limonin effect on PEC number by UNINOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

GROUP	N	Subset				
		1	2	3	4	5
Control	12	188.7500				
Buffer	12	202.5000				
Limonin5ppm	12	229.5833				
Limonin10ppm	12	249.1667	249.1667			
Limonin20ppm	12		298.7500			
Limonin50ppm	12			453.7500		
Limonin100ppm	12				708.7500	
Limonin200ppm	12					787.5000
Sig.		.075	.262	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares
The error term is Mean Square (Error) = 1626.018.

Table 8A Homogenous subset of group of mice fed with different concentration of limonin effect on PP by UNINOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

GROUP	N	Subset					
		1	2	3	4	5	6
Control	12	12.121667					
Buffer	12		38.687500				
Limonin5ppm	12		41.025833				
Limonin10ppm	12		43.941667				
Limonin20ppm	12			62.330000			
Limonin50ppm	12				79.215833		
Limonin100ppm	12					94.910833	
Limonin200ppm	12						107.966667
Sig.		1.000	.056	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares
The error term is Mean Square (Error) = 11.465.

Table 9A Homogenous subset of group of mice fed with different concentration of limonin effect on PI by UNINOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

GROUP	N	Subset						
		1	2	3	4	5	6	7
Control	12	.189933						
Buffer	12		.515108					
Limonin5ppm	12		.565108	.565108				
Limonin10ppm	12			.608267				
Limonin20ppm	12				.780833			
Limonin50ppm	12					.945242		
Limonin100ppm	12						1.255875	
Limonin200ppm	12							1.406992
Sig.		1.000	.738	.859	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares
The error term is Mean Square (Error) = 3.456E-03.

Table 10A Homogenous subset of day of limonin administration effect on PEC by MANOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

DAYS	N	Subset			MSE
		1	2	3	
2	32	355.4688			
4	32		390.0000		
6	32			424.0625	
Sig		1.000	1.000	1.000	393.663
2	16	433.7500			
4	16		471.8750		
6	16			510.0000	
Sig		1.000	1.000	1.000	466.319

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares Alpha = .05.

Table 11A Homogenous subset of day of limonin administration effect on PP by MANOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

DAYS	N	Subset			MSE
		1	2	3	
2	32	57.922813			
4	32		59.033437		
6	32			63.118750	
Sig		1.000	1.000	1.000	2.168
2	16	60.655000			
4	16	61.724375			
6	16		67.885625		
Sig		1.000	1.000	1.000	2.712

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares Alpha = .05.

Table 12A Homogenous subset of day of limonin administration effect on PI by MANOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

DAYS	N	Subset			MSE
		1	2	3	
2	32	0.743894			
4	32		0.769650		
6	32			0.836716	
Sig		1.000	1.000	1.000	0.0003
2	16	.781925			
4	16		.820294		
6	16			.923712	
Sig		1.000	1.000	1.000	0.0003

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares Alpha = .05.

4. Effect of Limonin from Lime Seeds on Total White Blood Cell Count

Table 13A The tests of effects of groups of mice fed with different concentration of limonin on total white blood cell count (5 large squares) after different days of limonin administration by UNIANOVA

Dependent Variable: total WBC count						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	28929.565	79	366.197	49.979	.000	
Intercept	2765340.602	1	2765340.602	377413.560	.000	
GROUP	27556.315	7	3936.616	537.269	.000	
DAY	648.169	9	72.019	9.829	.000	
GROUP * DAY	725.081	63	11.509	1.571	.006	
Error	2930.833	400	7.327			
Total	2797201.000	480				
Corrected Total	31860.398	479				

1 R Squared = .908 (Adjusted R Squared = .890)

Table 14A Homogenous subset of group of mice fed with different concentration of limonin effect on total WBC count by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = 0.05$)

GROUP	N	Subset					
		1	2	3	4	5	6
Control	60	63.4500					
Buffer	60		71.5000				
Limonin5ppm	60		72.5667				
Limonin10ppm	60		73.2833	73.2833			
Limonin20ppm	60			74.5000			
Limonin50ppm	60				77.7333		
Limonin100ppm	60					84.7333	
Limonin200ppm	60						89.4500
Sig.		1.000	.075	.534	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square (Error) = 7.327.

Table 15A Homogenous subset of group of mice fed with different concentration of limonin effect on total WBC count after different days of limonin administration by Scheffe's post hoc test by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test

GROUP	N	Subset					
		1	2	3	4	5	6
Control	6	62.3333					
Buffer	6		70.5000				
Limonin5ppm	6		71.1667				
Limonin10ppm	6		71.8333				
Limonin20ppm	6		73.1667				
Limonin50ppm	6		76.3333				
Limonin100ppm	6			82.6667			

Limonin200ppm	6			84.5000		
Sig.		1.000	.066	.982		
The error term for Days 3 is Mean Square (Error) = 6.938.						
GROUP	N	Subset				
		1	2	3	4	5
Control	6	63.6667				
Buffer	6		70.1667			
Limonin5ppm	6		71.0000			
Limonin10ppm	6		72.0000	72.0000		
Limonin20ppm	6		73.3333	73.3333		
Limonin50ppm	6			76.6667	76.6667	
Limonin100ppm	6				80.5000	80.5000
Limonin200ppm	6					84.1667
Sig.		1.000	.642	.163	.394	.453
The error term for Days 6 is Mean Square (Error) = 5.829.						
Control	6	63.8333				
Buffer	6		70.1667			
Limonin10ppm	6		72.0000			
Limonin5ppm	6		72.3333			
Limonin20ppm	6		74.8333	74.8333		
Limonin50ppm	6			78.6667	78.6667	
Limonin100ppm	6				83.5000	83.5000
Limonin200ppm	6					86.5000
Sig.		1.000	.286	.544	.244	.808
The error term for Days 9 is Mean Square (Error) = 7.304.						
Control	6	63.1667				
Buffer	6		72.6667			
Limonin5ppm	6		73.5000	73.5000		
Limonin10ppm	6		74.1667	74.1667		
Limonin20ppm	6		76.5000	76.5000		
Limonin50ppm	6			79.3333		
Limonin100ppm	6				90.0000	
Limonin200ppm	6				94.0000	
Sig.		1.000	.634	.130	.582	
The error term for Days 12 is Mean Square (Error) = 8.433.						
Control	6	63.8333				
Buffer	6		74.0000			
Limonin5ppm	6		74.6667			
Limonin10ppm	6		74.6667			
Limonin20ppm	6		75.8333			
Limonin50ppm	6		77.6667			
Limonin100ppm	6			84.3333		
Limonin200ppm	6				90.8333	

Limonin200ppm	6			93.0000		
Sig.		1.000	.055	1.000	1.000	
The error term for Days 27 is Mean Square (Error) = 5.542.						
Control	6	62.3333				
Buffer	6		71.0000			
Limonin5ppm	6		72.5000			
Limonin10ppm	6		73.1667			
Limonin20ppm	6		74.5000	74.5000		
Limonin50ppm	6			79.5000		
Limonin100ppm	6				86.0000	
Limonin200ppm	6					92.8333
Sig.		1.000	.659	.209	1.000	1.000

The error term for Days 30 is Mean Square (Error) = 7.338.

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares

a Uses Harmonic Mean Sample Size = 6.000.

b Alpha = .05.

5. Effect of Limonin from Lime Seeds on WBC-Differentiation

Table 16A The tests of effects of groups of mice fed with 0.5 ml/day/mouse of PBS, 100 ppm, and 200 ppm of limonin and untreated group and day after limonin administration (day 9, 12, and 15) on WBC-differentiation (n=6/group)(in percentage) by MANOVA

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Neutrophil	521.278a	11	47.389	20.388	.000
	Lymphocyte	350.515b	11	31.865	15.134	.000
	Monocyte	18.492c	11	1.681	4.454	.000
Intercept	Neutrophil	239782.742	1	239782.742	103159.699	.000
	Lymphocyte	112790.334	1	112790.334	53568.294	.000
	Monocyte	529.263	1	529.263	1402.353	.000
GROUP	Neutrophil	347.639	3	115.880	49.854	.000
	Lymphocyte	239.589	3	79.863	37.930	.000
	Monocyte	10.526	3	3.509	9.296	.000
DAY	Neutrophil	54.553	2	27.276	11.735	.000
	Lymphocyte	30.529	2	15.265	7.250	.002
	Monocyte	3.529	2	1.764	4.675	.013
GROUP * DAY	Neutrophil	119.087	6	19.848	8.539	.000
	Lymphocyte	80.396	6	13.399	6.364	.000
	Monocyte	4.438	6	.740	1.960	.086
Error	Neutrophil	139.463	60	2.324		
	Lymphocyte	126.333	60	2.106		
	Monocyte	22.645	60	.377		
Total	Neutrophil	240443.483	72			
	Lymphocyte	113267.182	72			
	Monocyte	570.400	72			
Corrected Total	Neutrophil	660.741	71			
	Lymphocyte	476.848	71			
	Monocyte	41.137	71			

a R Squared = .789 (Adjusted R Squared = .750)

b R Squared = .735 (Adjusted R Squared = .686)

c R Squared = .450 (Adjusted R Squared = .349)

Table 17A Homogenous subset of group of mice fed with different concentration of limonin effect on WBC-differentiate count after the 9th,12th, and 15th day of limonin administration by MANOVA with Scheffe's Multiple Contrasts Post Hoc test

Dependent Variable	GROUP	N	Subset				MSE
			1	2	3	4	
Neutrophil	Limoin200	18	54.7056				
	Limoin100	18		56.9594			
	Buffer	18			58.4256		
	Control	18				60.7450	
	Sig.			1.000	1.000	1.000	1.000
Lymphocyte	Control	18	37.0017				
	Buffer	18		39.1200			
	Limoin100	18		40.1383			
	Limoin200	18			42.0578		
	Sig.			1.000	.230	1.000	
Monocyte	Control	18	2.2550				
Dependent Variable	GROUP	N	Subset				MSE
			1	2	3	4	
Monocyte	Buffer	18	2.4539	2.4539			
	Limoin100	18		2.9011	2.9011		
	Limoin200	18			3.2350		
	Sig.		.815	.201	.454		.377

Based on Type III Sum of Squares

a Uses Harmonic Mean Sample Size = 18.000.

b Alpha = .05.

Table 18A Homogenous subset of day after different concentration of limonin administration on WBC differentiate count by UNINOVA with Scheffe's Multiple Contrasts Post Hoc test (significant at the .05 level)

Dependent Variable	DAYS	N	Subset		MSE
			1	2	
Neutrophil	15	24	44.1667		
	9	24	44.7083	44.7083	
	12	24		45.5000	
Sig.			.510	.242	2.586
Lymphocyte	9	24	29.4583		
	15	24		31.7500	
	12	24		32.1250	
Sig.			1.000	.766	3.144
Monocyte	9	24	1.8333		
	12	24		2.3333	
	15	24		2.3333	
Sig.			1.000	1.000	.250

Based on Type III Sum of Squares

a Uses Harmonic Mean Sample Size = 24.000.

b Alpha = .05.

6. Effect of Limonin from Lime Seeds on Specific Antigen-Antibody Titer

Table 19A The tests of effects of groups of mice fed with different concentration of limonin and day of limonin administration on specific antigen antibody titer by UNINOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3451.831	89	38.785	152.429	.000
Intercept	11106.669	1	11106.669	43650.662	.000
GROUP	1694.081	8	211.760	832.245	.000
DAY	1496.720	9	166.302	653.590	.000
GROUP * DAY	261.030	72	3.625	14.248	.000
Error	114.500	450	.254		
Total	14673.000	540			
Corrected Total	3566.331	539			

1 R Squared = .968 (Adjusted R Squared = .962)

Table 20A Homogenous subset of groups of mice fed with different concentration of limonin effect on specific antigen-antibody titer by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test

GROUP	N	Subset					
		1	2	3	4	5	6
Untreat+Unimm	60	.45					
Untreat+Imm	60		4.10				
PBS	60		4.20				
Limonin5ppm	60		4.32	4.32			
Limonin10ppm	60			4.60	4.60		
Limonin20ppm	60			4.62	4.62		
Limonin50ppm	60				4.90		
Limonin100ppm	60					6.25	
Limonin200ppm	60						7.38
Sig.		1.000	.699	.228	.228	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares, the error term is Mean Square (Error) = .254.

1 Uses Harmonic Mean Sample Size = 60.000.

2 Alpha = .05.

Table 21A Homogenous subsets of groups of mice fed with different concentration of limonin effect and day after of limonin administration on specific antigen-antibody titer by UNIANOVA with Scheffe's Multiple Contrasts Post Hoc test ($\alpha = .05$)

GROUP	N	Subset				
		1	2	3	4	5
Untreat+Unimm	6	.00				
Untreat+Imm	6	.00				
PBS	6	.00				
Limonin5ppm	6	.00				
Limonin10ppm	6	.00				
Limonin20ppm	6	.00				
Limonin50ppm	6	.00				
Limonin100ppm	6	.00				
Limonin200ppm	6	.00				
Sig.		1.000				

The error term for Days 3 is Mean Square (Error) = .000.

Untreat+Unimm	6	.33				
Untreat+Imm	6		3.50			
PBS	6		3.50			
Limonin5ppm	6		3.50			
Limonin50ppm	6		3.67			
Limonin20ppm	6		3.83	3.83		
Limonin10ppm	6		3.83	3.83		
Limonin100ppm	6			5.33	5.33	
Limonin200ppm	6				6.67	
Sig.		1.000	.999	.109	.225	

The error term for Days 6 is Mean Square (Error) = .478.

GROUP	N	Subset				
		1	2	3	4	5
Untreat+Unimm	6	.33				
Untreat+Imm	6		4.50			
PBS	6		4.67			
Limonin5ppm	6		4.67			
Limonin20ppm	6		4.83			
Limonin50ppm	6		4.83			
Limonin10ppm	6		5.00			
Limonin100ppm	6			6.67		
Limonin200ppm	6			7.83		
Sig.		1.000	.930	.065		
The error term for Days 9 is Mean Square (Error) = .252.						
Untreat+Unimm	6	.67				
Untreat+Imm	6		4.67			
PBS	6		4.83			
Limonin5ppm	6		4.83			
Limonin10ppm	6		5.33			
Limonin20ppm	6		5.50			
Limonin50ppm	6		5.83			
Limonin100ppm	6			7.83		
Limonin200ppm	6				9.67	
Sig.		1.000	.286	1.000	1.000	
The error term for Days 12 is Mean Square (Error) = .404.						
Untreat+Unimm	6	.50				
Untreat+Imm	6		5.17			
PBS	6		5.50			
Limonin5ppm	6		5.83	5.83		
Limonin10ppm	6		5.83	5.83		
Limonin20ppm	6		6.00	6.00		
Limonin50ppm	6			6.83		
Limonin100ppm	6				8.50	
Limonin200ppm	6					9.67
Sig.		1.000	.279	.094	1.000	1.000
The error term for Days 15 is Mean Square (Error) = .204.						
Untreat+Unimm	6	.50				
Untreat+Imm	6		5.00			
PBS	6		5.50			
Limonin5ppm	6		5.67			
Limonin10ppm	6		6.00	6.00		
Limonin20ppm	6		6.00	6.00		
Limonin50ppm	6			7.00		
Limonin100ppm	6				8.83	
Limonin200ppm	6				9.67	
Sig.		1.000	.150	.150	.371	
The error term for Days 18 is Mean Square (Error) = .233.						
Untreat+Unimm	6	.67				

GROUP	N	Subset				
		1	2	3	4	5
Untreat+Imm	6		4.83			
PBS	6		4.83			
Limonin5ppm	6		5.50	5.50		
Limonin10ppm	6		5.50	5.50		
Limonin20ppm	6		5.67	5.67		
Limonin50ppm	6			6.00		
Limonin100ppm	6				7.17	
Limonin200ppm	6					8.67
Sig.		1.000	.302	.888	1.000	1.000
The error term for Days 21 is Mean Square (Error) = .211.						
Untreat+Unimm	6	.50				
Untreat+Imm	6		4.83			
PBS	6		4.83			
Limonin5ppm	6		4.83			
Limonin10ppm	6		5.50	5.50		
Limonin20ppm	6		5.50	5.50		
Limonin50ppm	6		5.50	5.50		
Limonin100ppm	6			6.67	6.67	
Limonin200ppm	6				8.00	
Sig.		1.000	.868	.202	.084	
The error term for Days 24 is Mean Square (Error) = .352.						
Untreat+Unimm	6	.50				
Untreat+Imm	6		4.50			
PBS	6		4.50			
Limonin5ppm	6		4.50			
Limonin10ppm	6		4.67			
Limonin20ppm	6		4.83			
Limonin50ppm	6		5.00			
Limonin100ppm	6			6.17		
Limonin200ppm	6			7.17		
Sig.		1.000	.897	.121		
The error term for Days 27 is Mean Square (Error) = .219.						
Untreat+Unimm	6	.50				
Untreat+Imm	6		3.83			
PBS	6		4.00			
Limonin5ppm	6		3.83			
Limonin20ppm	6		4.00			
Limonin10ppm	6		4.33	4.33		
Limonin50ppm	6		4.33	4.33		
Limonin100ppm	6			5.33		
Limonin200ppm	6				6.50	
Sig.		1.000	.859	.076	1.000	
The error term for Days 30 is Mean Square (Error) = .193.						

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares, uses Harmonic Mean Sample Size = 6.000.

6. Effect of Processing of Lime Juices on Limonin Content

Table 22A The tests of effects of processing of lime juice on limonin content by UNINOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	216.76a5	5	43.353	7042.912	.000
Intercept	5003.749	1	5003.749	812883.382	.000
PROCESS	216.765	5	43.353	7042.912	.000
Error	.111	18	6.156E-03		
Total	5220.625	24			
Corrected Total	216.876	23			

a R Squared = .999 (Adjusted R Squared = .999)

7. Effect of Processing of Lime Juices on Total White Blood Cell Count

Table 23A The tests of effects of groups of mice fed with different processing of lime juice and day of lime juice administration on total white blood cell count by UNIANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	84869.315	79	1074.295	121.762	.000
Intercept	3448138.519	1	3448138.519	390816.172	.000
GROUP	83373.931	7	11910.562	1349.957	.000
DAY	687.210	9	76.357	8.654	.000
GROUP * DAY	808.173	63	12.828	1.454	.019
Error	3529.167	400	8.823		
Total	3536537.000	480			
Corrected Total	88398.481	479			

1 R Squared = .960 (Adjusted R Squared = .952)

Table 24A Homogenous subset of groups of mice fed with different processing of lime on total white blood cell count after different days of lime juice administration by UNIANOVA with Scheffe's Multiple Contrast Post Hoc test

GROUP	N	Subset					
		1	2	3	4	5	6
conUntreat	6	62.3333					
conPBS	6		70.5000				
hand+heat	6			77.1667			
Machine+heat	6			81.1667	81.1667		
hand+freeze	6				84.3333	84.3333	
Machine+freez	6					89.5000	
e							
Hand	6						96.1667
Machine	6						100.6667
Sig.		1.000	1.000	.539	.795	.213	.382

The error term for Days 3 is Mean Square (Error) = 7.887.

conUntreat	6	63.6667				
conPBS	6		70.1667			
hand+heat	6			77.0000		
machine+heat	6			81.5000	81.5000	
hand+freeze	6				83.8333	83.8333

machine+freez	6						90.0000
e							
Hand	6						96.8333
Machine	6						102.8333
	N	Subset					
GROUP		1	2	3	4	5	6
Sig.		1.000	1.000	.366	.948	.064	.079
The error term for Days 6 is Mean Square (Error) = 7.704.							
conUntreat	6	63.8333					
conPBS	6	70.1667					
hand+heat	6		78.8333				
machine+heat	6		83.0000				
hand+freeze	6		84.1667				
machine+freez	6			92.0000			
e							
Hand	6			97.6667			
machine	6				104.6667		
Sig.		.065	.197	.140	1.000		
The error term for Days 9 is Mean Square (Error) = 8.150.							
conUntreat	6	63.1667					
conPBS	6		72.6667				
hand+heat	6			79.6667			
machine+heat	6			84.5000			
hand+freeze	6			85.5000			
machine+freez	6				92.5000		
e							
Hand	6				98.6667		
machine	6					113.6667	
Sig.		1.000	1.000	.169	.121	1.000	
The error term for Days 12 is Mean Square (Error) = 9.217.							
conUntreat	6	63.8333					
conPBS	6		74.0000				
hand+heat	6			81.8333			
machine+heat	6			83.5000			
hand+freeze	6			87.5000	87.5000		
machine+freez	6				93.5000	93.5000	
e							
Hand	6					99.0000	
machine	6						110.6667
Sig.		1.000	1.000	.188	.135	.219	1.000
The error term for Days 15 is Mean Square (Error) = 9.037.							
conUntreat	6	64.1667					
conPBS	6		71.3333				
hand+heat	6			80.5000			
machine+heat	6			83.8333			
hand+freeze	6			86.0000			
machine+freez	6				93.0000		
e							
Hand	6				98.3333		
machine	6					109.3333	
Sig.		1.000	1.000	.233	.269	1.000	
The error term for Days 18 is Mean Square (Error) = 9.279.							
conUntreat	6	63.5000					
conPBS	6		72.1667				
hand+heat	6			79.6667			

machine+heat	6			81.3333			
hand+freeze	6			85.3333	85.3333		
machine+freez	6				92.3333	92.3333	
e							
Hand	6					94.8333	
machine	6						109.3333
Sig.		1.000	1.000	.213	.054	.957	1.000

The error term for Days 21 is Mean Square (Error) = 9.496.

conUntreat	6	64.0000					
	N	Subset					
GROUP		1	2	3	4	5	6
conPBS	6		72.0000				
hand+heat	6			79.6667			
machine+heat	6			81.5000			
hand+freeze	6			85.8333	85.8333		
machine+freez	6				92.5000	92.5000	
e							
Hand	6					94.3333	
machine	6						106.5000
Sig.		1.000	1.000	.180	.113	.995	1.000

The error term for Days 24 is Mean Square (Error) = 10.550.

conUntreat	6	63.6667					
conPBS	6		71.0000				
hand+heat	6			78.6667			
machine+heat	6			79.5000			
hand+freeze	6			85.3333			
machine+freez	6				92.5000		
e							
Hand	6				95.3333		
machine	6					108.1667	
Sig.		1.000	1.000	.073	.915	1.000	

The error term for Days 27 is Mean Square (Error) = 9.329.

conUntreat	6	62.3333					
conPBS	6		71.0000				
hand+heat	6			79.3333			
machine+heat	6			80.5000	80.5000		
hand+freeze	6				85.6667		
machine+freez	6					92.6667	
e							
Hand	6					96.8333	
machine	6						110.5000
Sig.		1.000	1.000	.999	.192	.458	1.000

The error term for Days 30 is Mean Square (Error) = 7.579.

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares
a Uses Harmonic Mean Sample Size = 6.000.

b Alpha = .05.

8. Effect of Processing of Lime Juices on Specific Antigen-Antibody Titer

Table 25A The tests of effects of groups of mice fed with different processing of lime juice on specific antigen antibody titer by UNIANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3590.592	79	45.451	122.015	.000
Intercept	14896.408	1	14896.408	39990.358	.000
GROUP	2380.592	7	340.085	912.979	.000

DAY	1030.925	9	114.547	307.509	.000
GROUP * DAY	179.075	63	2.842	7.631	.000
Error	149.000	400	.372		
Total	18636.000	480			
Corrected Total	3739.592	479			

1 R Squared = .960 (Adjusted R Squared = .952)

Table 26A Homogenous subset of groups of mice fed with different processing of lime juice on specific antigen-antibody titer by UNINOVA with Scheffe's Multiple Contrast Post Hoc test

GROUP	N	Subset					
		1	2	3	4	5	6
ConUntreat+Unimm	60	.45					
ConUntreat+Imm	60		4.10				
Hand+Heat	60			5.35			
Machine+Heat	60				6.10		
Hand+Freeze	60				6.42		
Machine+Freeze	60					6.95	
Hand	60					7.37	
Machine	60						7.83
Sig.		1.000	1.000	1.000	.329	.054	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares, the error term is Mean Square (Error) = .372.

1 Uses Harmonic Mean Sample Size = 60.000.

2 Alpha = .05.

Table 27A Homogenous subset of groups of mice fed with different processing of lime juice and day of lime juice administration on specific antigen-antibody titer by MANOVA with Scheffe's Multiple Contrast Post Hoc test ($\alpha = .05$)

GROUP	N	Subset				
		1	2	3	4	5
ConUntreat+Unimm	6	.00				
ConUntreat+Imm	6	.00				
Hand+Heat	6	.00				
Machine+Heat	6	.67	.67			
Hand+Freeze	6		2.00	2.00		
Machine+Freeze	6			2.50	2.50	
Hand	6				3.67	
Machine	6				3.67	
Sig.		.835	.084	.960	.193	

The error term for Days 3 is Mean Square (Error) = .387.

ConUntreat+Unimm	6	.33				
ConUntreat+Imm	6		3.50			
Hand+Heat	6			5.67		
Hand+Freeze	6			5.83		
Machine+Heat	6			6.00	6.00	
Machine+Freeze	6			6.50	6.50	6.50
Hand	6				7.33	7.33
Machine	6					7.67
Sig.		1.000	1.000	.604	.078	.182

The error term for Days 6 is Mean Square (Error) = .379.

ConUntreat+Unimm	6	.33
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m						
ConUntreat+Imm	6		4.50			
Machine+Heat	6		6.00	6.00		
The error term for Days 9 is Mean Square (Error) = .654.						
	N	Subset				
GROUP		1	2	3	4	5
Hand+Heat	6		6.17	6.17		
Hand+Freeze	6			6.83	6.83	
Machine+Freeze	6			7.83	7.83	7.83
Hand	6				8.67	8.67
Machine	6					9.50
Sig.		1.000	.110	.055	.055	.110
The error term for Days 12 is Mean Square (Error) = .600.						
ConUntreat+Unim	6	.67				
m						
ConUntreat+Imm	6		4.83			
Hand+Heat	6			6.83		
Machine+Heat	6			7.50	7.50	
Hand+Freeze	6			7.67	7.67	
Machine+Freeze	6			8.50	8.50	8.50
Hand	6				8.67	8.67
Machine	6					9.67
Sig.		1.000	1.000	.082	.465	.465
The error term for Days 15 is Mean Square (Error) = .254.						
ConUntreat+Unim	6	.50				
m						
ConUntreat+Imm	6		5.17			
Hand+Heat	6			6.83		
Hand+Freeze	6			7.50	7.50	
Machine+Heat	6			7.67	7.67	
Hand	6				8.33	8.33
Machine+Freeze	6				8.50	8.50
Machine	6					9.33
Sig.		1.000	1.000	.341	.140	.140
The error term for Days 18 is Mean Square (Error) = .275.						
ConUntreat+Unim	6	.67				
m						
ConUntreat+Imm	6		5.00			
Hand+Heat	6			6.83		
Machine+Heat	6			7.83	7.83	
Hand+Freeze	6			7.83	7.83	
Hand	6				8.17	
Machine+Freeze	6				8.17	
Machine	6				8.67	
Sig.		1.000	1.000	.176	.393	
The error term for Days 18 is Mean Square (Error) = .275.						
ConUntreat+Unim	6	.67				
m						

ConUntreat+Imm	6	4.83			
ConUntreat+Imm	6	4.83			
Hand+Heat	6		6.33		
Machine+Heat	6		7.00	7.00	
Hand+Freeze	6		7.00	7.00	
Machine+Freeze	6			7.67	7.67
Hand	6			7.83	7.83
Machine	6				8.50

GROUP	N	Subset				
		1	2	3	4	5
Sig.		1.000	1.000	.686	.403	.403

The error term for Days 21 is Mean Square (Error) = .279.

ConUntreat+Unim	6	.50			
m					
ConUntreat+Imm	6		4.83		
Hand+Heat	6		5.33		
Machine+Heat	6			6.83	
Hand+Freeze	6			7.00	
Machine+Freeze	6			7.00	
Hand	6			7.50	
Machine	6			7.83	
Sig.		1.000	.899	.169	

The error term for Days 24 is Mean Square (Error) = .271.

ConUntreat+Unim	6	.50			
m					
ConUntreat+Imm	6		4.50		
Hand+Heat	6		4.83		
Machine+Heat	6			6.17	
Machine+Freeze	6			6.67	
Hand+Freeze	6			6.83	
Hand	6			7.00	
Machine	6			7.00	
Sig.		1.000	.993	.496	

The error term for Days 27 is Mean Square (Error) = .321.

ConUntreat+Unim	6	.50			
m					
ConUntreat+Imm	6		3.83		
Hand+Heat	6		4.67	4.67	
Machine+Heat	6			5.33	5.33
Hand+Freeze	6			5.67	5.67
Machine+Freeze	6				6.17
Hand	6				6.50
Machine	6				6.50
Sig.		1.000	.460	.229	.092

The error term for Days 30 is Mean Square (Error) = .304.

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares

- a Uses Harmonic Mean Sample Size = 6.000.
- b Alpha = .05.

APPENDIX 2
CROMATOGRAMS

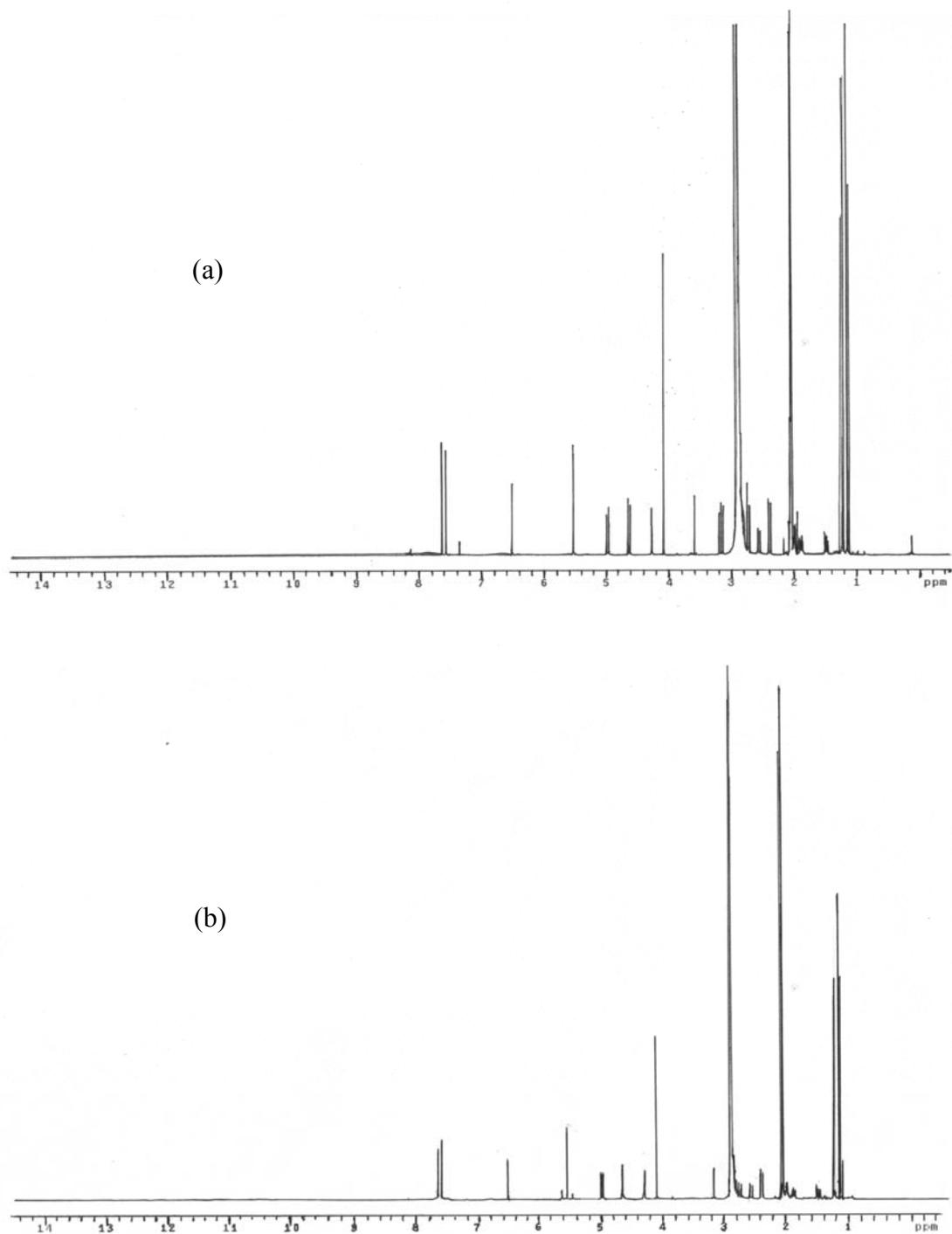


Figure 1B ^1H -NMR-spectrum of (a) limonin from lime seeds (b) standard limonin (Sigma®)

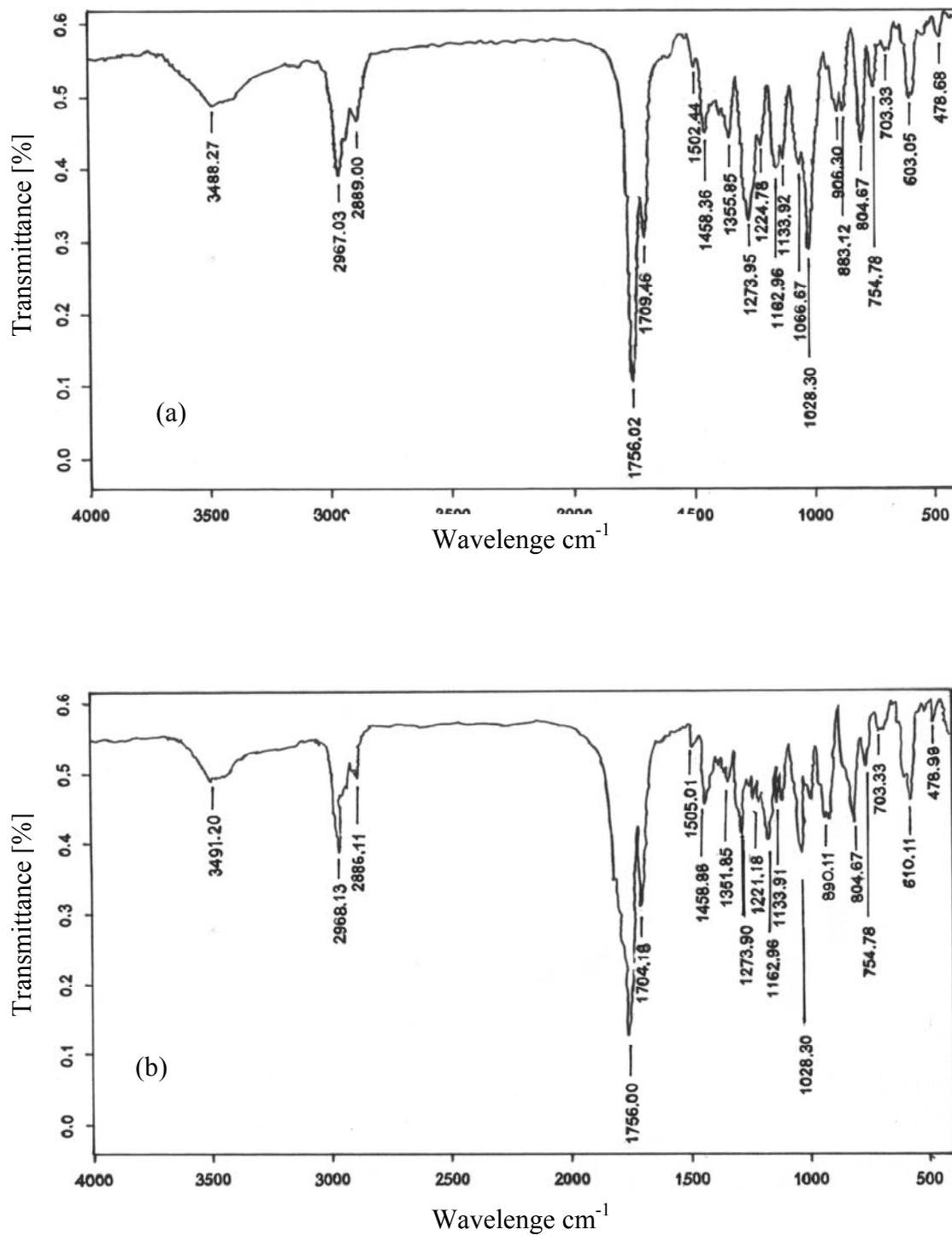


Figure 2B IR-spectrum of limonin (a) limonin from lime seeds (b) standard limonin (Sigma®)

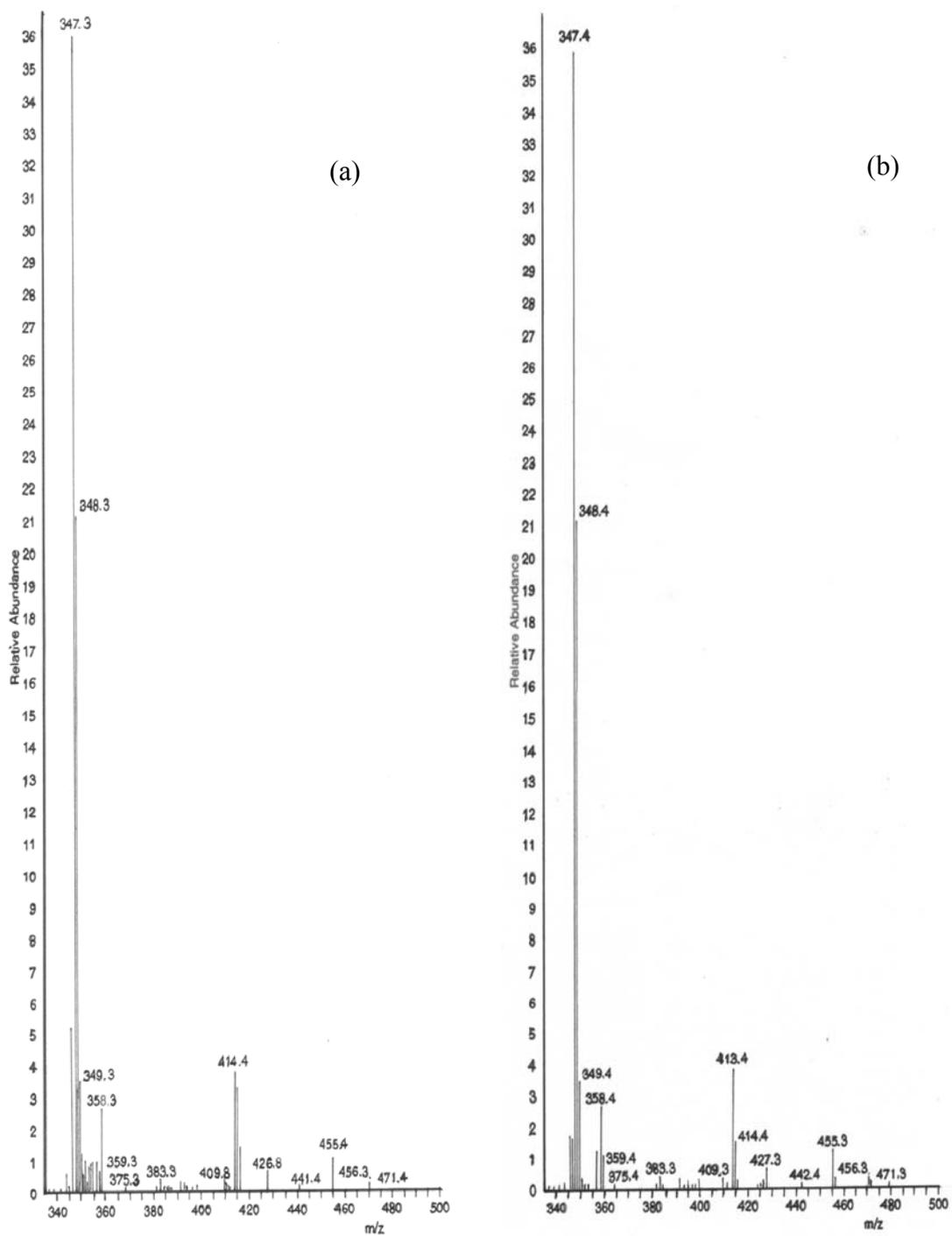


Figure 3B Mass spectrum of (a) limonin from lime seeds (b) standard limonin (Sigma®)

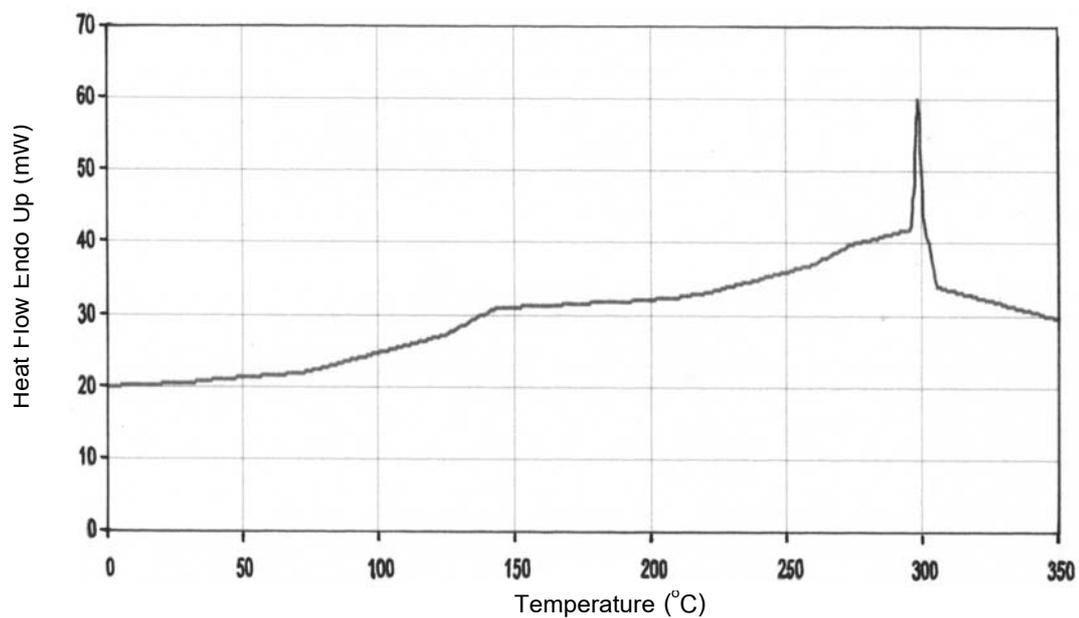


Figure 4B Thermogram of limonin

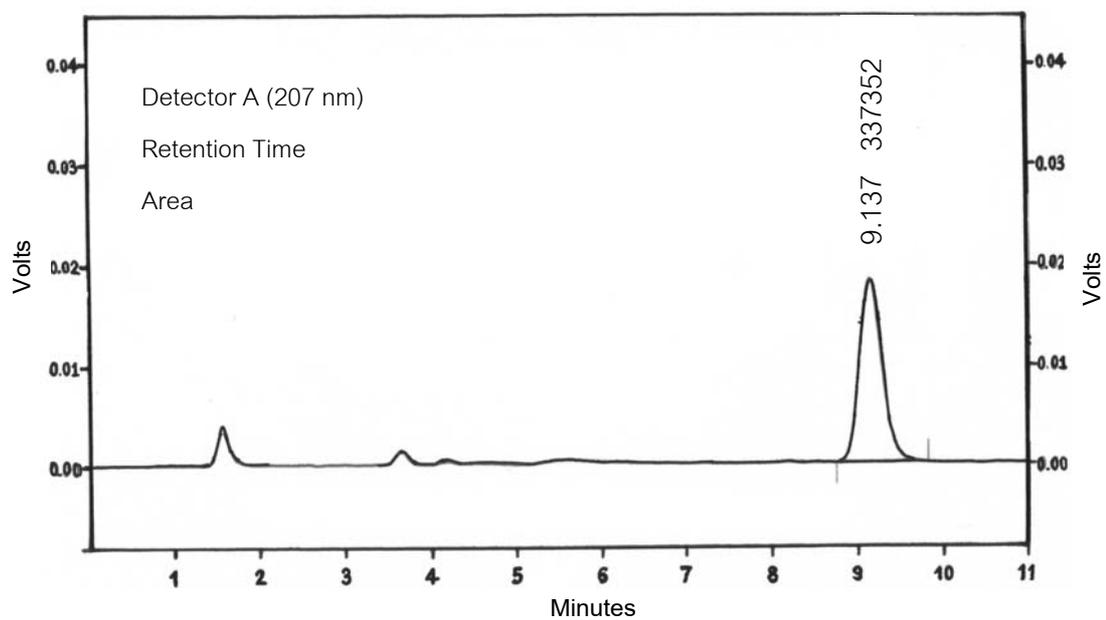


Figure 5B HPLC-chromatogram of limonin in solvent of acetonitrile and water, 40:60 v/v

Table 4C Limonin used for different temperature (45°C, 70°C, 80°C) with 70%RH degradation and its remaining concentration at various time (n = 3)

Time (days)	45°C		70°C		80°C	
	%D/D ₀	ln %D/D ₀	%D/D ₀	ln %D/D ₀	%D/D ₀	ln %D/D ₀
10	100.86±0.18	4.6137±0.0018	100.10±0.25	4.6062±0.0025	±0.27	4.5400±0.0029
20	99.41±1.22	4.5992±0.0122	97.64±0.18	4.5813±0.0018	90.06±0.05	4.5005±0.0005
30	98.53±0.15	4.5903±0.0015	92.44±0.29	4.5265±0.0032	87.67±0.14	4.4736±0.0016
60	91.75±0.08	4.5190±0.0009	89.26±0.08	4.4915±0.0009	81.74±0.12	4.4036±0.0014
90	87.21±0.03	4.4683±0.0004	82.70±0.83	4.4152±0.0100	75.53±0.27	4.3246±0.0035
120	83.76±1.00	4.4279±0.0119	78.34±0.34	4.3611±0.0043	70.05±0.24	4.2492±0.0035
150	81.42±0.97	4.3996±0.0118	74.68±0.15	4.3132±0.0020	66.68±0.29	4.1998±0.0044
180	76.70±0.87	4.3398±0.0114	69.09±0.32	4.2354±0.0046	62.09±0.06	4.1286±0.0010
210	72.27±0.75	4.2804±0.0103	65.28±0.09	4.1786±0.0014	57.59±0.26	4.0533±0.0044
240	68.28±0.25	4.2236±0.0037	61.75±0.30	4.1231±0.0048	54.92±0.13	4.0059±0.0023
270	66.14±0.10	4.1917±0.0015	57.78±0.35	4.0567±0.0060	50.51±0.12	3.9221±0.0023
300	63.61±0.12	4.1527±0.0020	55.21±0.18	4.0111±0.0033	47.73±0.20	3.8655±0.0041
330	61.17±0.17	4.1136±0.0027	51.86±0.15	3.9485±0.0029	45.67±0.18	3.8214±0.0039
360	58.13±0.21	4.0626±0.0035	48.27±0.12	3.8768±0.0025	42.83±0.02	3.7572±0.0005

Table 5C PEC in mice fed with different concentration of limonin (calculated as $\times 10^4$ cells/ml \pm SD) after 2, 4, and 6 days limonin administration (n = 4)

Treatment	Cell/ml X10 ⁴		
	2 days	4 days.	6 days.
Untreated	175.00±10.80	186.25±17.97	205.00±16.83
PBS	197.50±17.08	200.00±14.72	210.00±12.25
Limonin5ppm	222.50±16.58	228.75±16.52	237.50±14.43
Limonin10ppm	227.50±18.48	251.25±19.31	268.75±17.97
Limonin20ppm	263.75±16.52	301.25±16.52	331.25±17.50
Limonin50ppm	395.00±20.82	451.25±20.16	515.00±19.15
Limonin100ppm	648.75±20.56	716.25±26.58	761.25±29.55
Limonin200ppm	713.75±29.26	785.00±26.46	863.75±25.62

Table 6C PP and PI (\pm SD) in mice fed with 0.5 ml/day/mouse of different concentration of limonin after 2, 4, and 6 days limonin administration (n = 4)

Mice fed with	Days	PP	PI
Untreated	2	12.26 \pm 1.56	0.1777 \pm 0.0100
	4	11.23 \pm 0.36	0.1831 \pm 0.0053
	6	12.88 \pm 1.05	0.2090 \pm 0.0141
PBS	2	37.02 \pm 1.26	0.4862 \pm 0.0054
	4	38.41 \pm 0.60	0.5157 \pm 0.0093
	6	40.64 \pm 0.86	0.5435 \pm 0.0097
Limonin 5 ppm	2	40.18 \pm 1.47	0.5553 \pm 0.0155
	4	40.93 \pm 2.04	0.5644 \pm 0.0226
	6	41.97 \pm 1.40	0.5757 \pm 0.0151
Limonin 10 ppm	2	43.60 \pm 0.93	0.6034 \pm 0.0125
	4	43.27 \pm 0.79	0.5993 \pm 0.0048
	6	44.96 \pm 0.42	0.6221 \pm 0.0031
Limonin 20 ppm	2	60.91 \pm 1.94	0.7581 \pm 0.0267
	4	61.97 \pm 0.96	0.7727 \pm 0.0086
	6	64.11 \pm 0.95	0.8117 \pm 0.0097
Limonin 50 ppm	2	76.07 \pm 1.82	0.9067 \pm 0.0163
	4	79.21 \pm 0.55	0.9397 \pm 0.0100
	6	82.37 \pm 0.61	0.9894 \pm 0.0106
Limonin 100 ppm	2	89.79 \pm 1.05	1.1671 \pm 0.0166
	4	91.91 \pm 1.16	1.2250 \pm 0.0048
	6	103.04 \pm 2.76	1.3756 \pm 0.0266
Limonin 200 ppm	2	103.57 \pm 1.00	1.2968 \pm 0.0072
	4	105.36 \pm 2.92	1.3574 \pm 0.0294
	6	114.98 \pm 2.57	1.5669 \pm 0.0410

Table 7C Total WBC count ($\times 10^4$ cell/ml \pm SD) in mice fed with different concentration of limonin on different days after limonin administration (n =6)

Treatment	3 Days	6	9	12	15	18	21	24	27	30
Untreated	311.67 ± 15.38	318.33 ± 12.91	319.17 ± 13.57	315.83 ± 19.34	319.17 ± 16.56	320.83 ± 15.30	317.50 ± 17.25	320.00 ± 19.24	318.33 ± 16.02	311.67 ± 15.38
PBS	352.50 ± 14.05	350.83 ± 12.42	350.83 ± 13.57	363.33 ± 13.66	370.00 ± 13.78	356.67 ± 17.80	360.83 ± 12.01	360.00 ± 13.04	355.00 ± 11.83	355.00 ± 12.65
Limoinin 5ppm	355.83 ± 8.01	355.00 ± 7.75	361.67 ± 12.11	367.50 ± 10.37	373.33 ± 8.16	363.33 ± 14.02	363.33 ± 11.69	365.00 ± 8.94	360.83 ± 8.01	362.50 ± 10.84
Limoinin 10ppm	359.17 9.17	360.00 ± 9.49	360.00 ± 9.49	370.83 ± 12.42	373.33 ± 10.80	369.17 ± 9.70	370.83 ± 6.65	366.67 ± 15.38	368.33 ± 8.16	365.83 ± 9.70
Limoinin 20ppm	365.83 ± 10.68	366.67 ± 7.53	374.17 ± 8.61	382.50 ± 8.22	379.17 ± 8.01	372.50 ± 13.69	375.00 ± 13.42	367.50 ± 12.14	369.17 ± 9.17	372.50 ± 15.08
Limoinin 50ppm	381.67 ± 12.91	383.33 ± 15.38	393.33 ± 19.92	396.67 ± 21.83	388.33 ± 16.02	390.83 ± 15.30	384.17 ± 15.30	389.17 ± 19.08	381.67 ± 14.38	397.50 ± 16.36
Limoinin 100ppm	413.33 ± 15.06	402.50 ± 12.14	417.50 ± 14.40	450.00 ± 12.25	421.67 ± 11.25	420.83 ± 12.42	425.00 ± 10.49	425.83 ± 15.63	430.00 ± 10.49	430.00 ± 15.17
Limoinin 200ppm	422.50 ± 17.25	420.83 ± 15.94	432.50 ± 13.32	470.00 ± 13.04	454.17 ± 19.34	442.50 ± 17.82	443.33 ± 12.11	457.50 ± 16.36	465.00 ± 13.42	464.17 ± 13.20

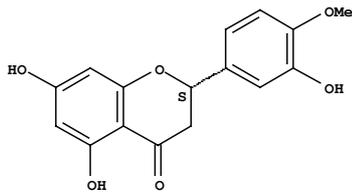
Table 8C Total WBC count ($\times 10^4$ cell/ml \pm SD) in mice fed with various processed lime juices on different days after lime juice administration (n = 6)

Group	3 days	6	9	12	15	18	21	24	27	30
Untreated	311.67 ± 15.38	318.33 ± 12.91	319.17 ± 13.57	315.83 ± 19.34	319.17 ± 16.56	320.83 ± 15.30	317.50 ± 17.25	320.00 ± 19.24	318.33 ± 16.02	311.67 ± 14.02
Hand	480.83 ± 13.20	484.17 ± 17.15	488.33 ± 17.51	493.33 ± 17.80	495.00 ± 15.49	491.67 ± 15.38	474.17 ± 15.30	471.67 ± 16.02	476.67 ± 18.35	484.17 ± 18.82
Machine	503.33 ± 13.29	514.17 ± 15.63	523.33 ± 13.29	568.33 ± 15.38	553.33 ± 16.33	546.67 ± 18.35	546.67 ± 18.62	532.50 ± 19.94	540.83 ± 17.72	552.50 ± 14.05
Hand +Heat	385.83 ± 13.20	385.00 ± 13.78	394.17 ± 15.30	398.33 ± 13.29	409.17 ± 14.29	402.50 ± 13.32	398.33 ± 13.66	398.33 ± 12.11	393.33 ± 13.66	396.67 ± 10.80
Machine +Heat	405.83 ± 13.57	407.50 ± 13.32	415.00 ± 14.14	422.50 ± 13.32	417.50 ± 13.32	419.17 ± 13.57	406.67 ± 18.07	407.50 ± 18.10	397.50 ± 15.08	402.50 ± 12.14
Hand +Freeze	421.67 ± 15.06	419.17 ± 12.81	420.83 ± 13.20	427.50 ± 15.08	437.50 ± 17.54	430.00 ± 13.04	426.67 ± 14.02	429.17 ± 15.30	426.67 ± 14.72	428.33 ± 13.29
Machine +Freeze	447.50 ± 14.40	450.00 ± 12.25	460.00 ± 13.04	462.50 ± 12.14	467.50 ± 12.14	465.00 ± 14.14	461.67 ± 12.91	462.50 ± 14.40	462.50 ± 13.69	463.33 ± 12.91

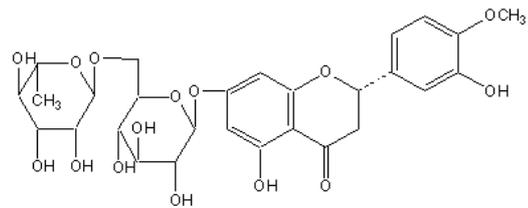
APPENDIX 4

SOME PHYTOCHEMICAL STRUCTURES IN LIME

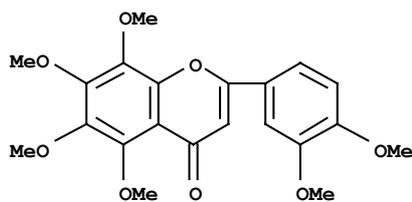
1. Flavonoids



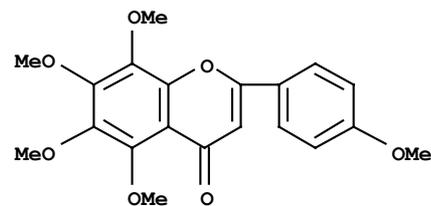
Hesperetin



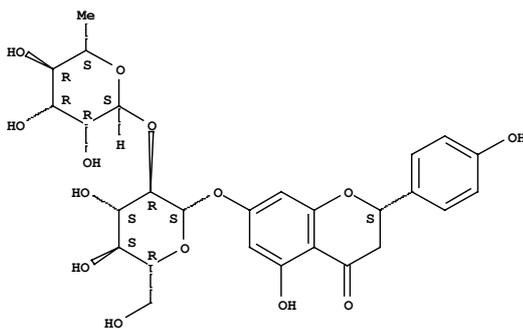
Hesperidin



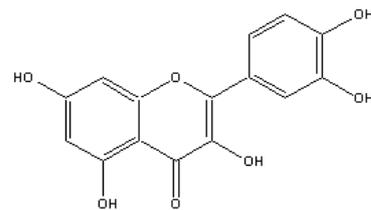
Nobiletin



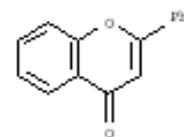
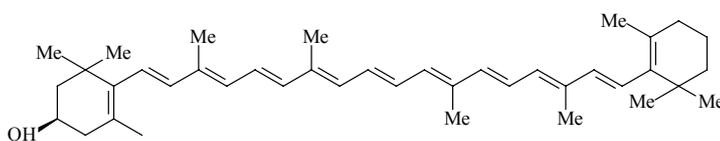
Tangeretin



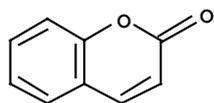
Naringin



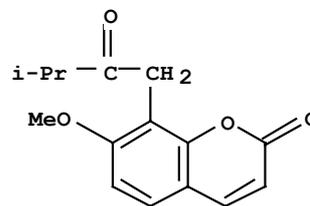
Quercetin



2. Coumarins

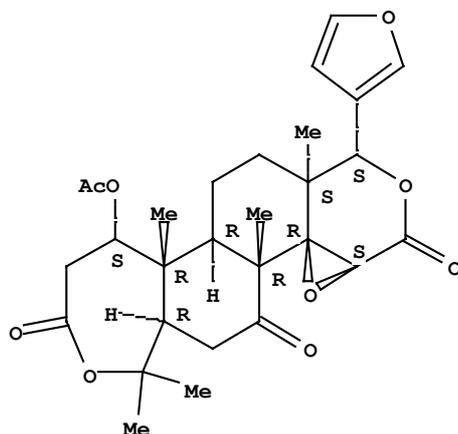


Coumarin

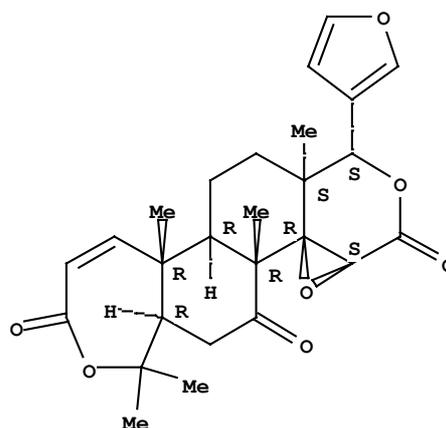


Auraptene

3. Limonoids

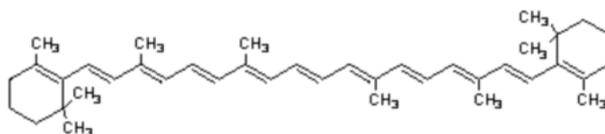
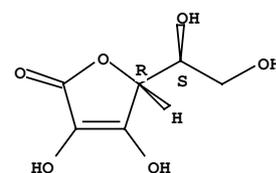


Nomilin



Obacunone

4. Carotenoids

 β -carotene

Ascorbic Acid (Vitamin C)