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APPENDIX A

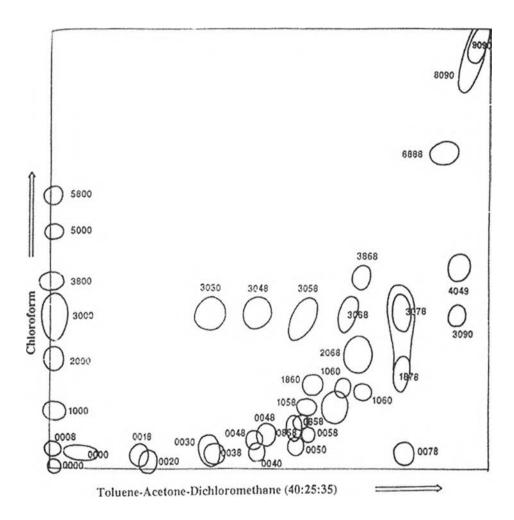


Figure 19: Two-dimensional thin layer chromatogram in Toluene-Acetone-Dichloromethane (40:25:35) and Chloroform of stem of *Derris reticulata* Craib.

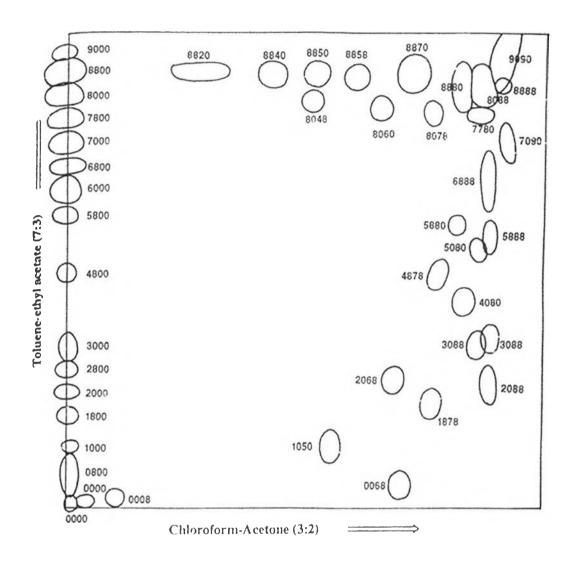


Figure 20: Two-dimensional thin layer chromatogram of stem in Chloroform-Acetone (3:2) and Toluene-ethyl acetate (7:3) of *Derris reticulata* Craib.

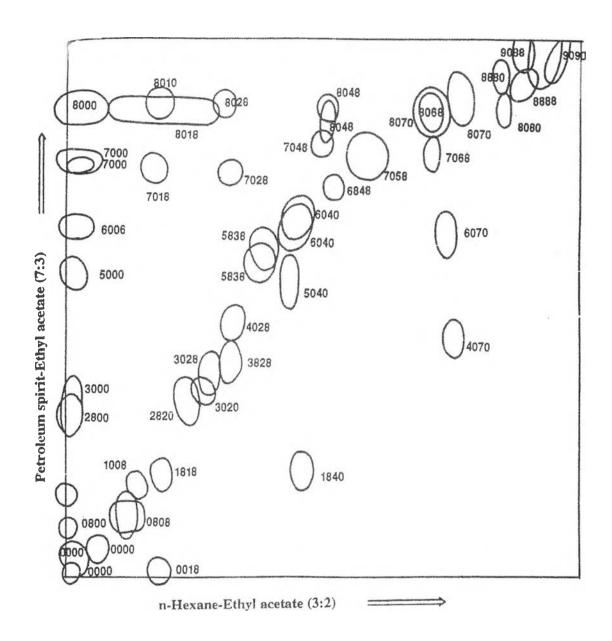


Figure 21: Two-dimensional thin layer chromatogram of stem in n-Hexane-Ethyl acetate (3:2) and Petroleum spirit-Ethyl acetate (7:3) of *Derris reticulata* Craib.

APPENDIX B

EXPERIMENT RESULTS

Table 2: Data of standard calibration curve of nitrite by Griess reaction

	Absorba		
concentration	nr	Mean	
(µM)	1	2	
0	0.072	0.072	0.072
1.531	0.085	0.088	0.0865
3.13	0.105	0.106	0.1055
6.25	0.136	0.143	0.1395
12.5	0.212	0.212	0.212
25	0.347	0.355	0.351
50	0.624	0.626	0.625
100	1.173	1.71	1.172

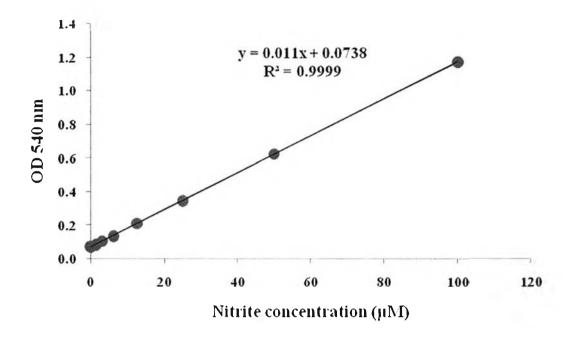


Figure 22: Standard nitrite calibration curve by Griess reaction

Table 3: Data of nitrite concentrations in experiments of nitric oxide production released from J774A.1 cells by Griess reaction (n=4).

Took as were assets		N	itrie concer			
Test compounds		1	2	3	4	Mean ± S.E.
0.2 % DMSO	32.54	31.23	28.97	32.65	31.35 ± 1.71	
DEX (10 μM)		15.73	16.49	11.20	13.89	14.33 ± 2.35
	6.25		32.50	27.57	29.53	30.62 ± 2.53
	12.5	28.11	30.03	25.16	27.26	27.64 ± 2.02
D. reticulata (µg/ml) - 2		24.44	25.69	21.23	21.78	23.29 ± 2.13
	50	18.42	18.84	14.07	15.39	16.68 ± 2.32
	100	6.96	8.78	7.68	7.88	7.83 ± 0.75

Table 4: Data of the effect on phagocytosis activity of the ethanol extract stem of *D*. *reticulata* (n=3).

The results were expressed as the percentage of inhibition over untreated control (mean \pm S.E), (n=3). *P<0.05 compared with the untreated control.

Toot compounds	% inhibit	tion of phag	Maan I C F		
Test compounds	•	1	2	3	Mean ± S.E.
DEX (10 μM)		3.95	6.13	4.48	4.99 ± 1.10
	25		1.92	2.34	3.08 ± 1.70
D.reticulata (µg/ml) - 5		23.10	27.17	26.80	25.69 ± 2.25
	100	60.07	60.13	62.15	60.78 ± 1.18

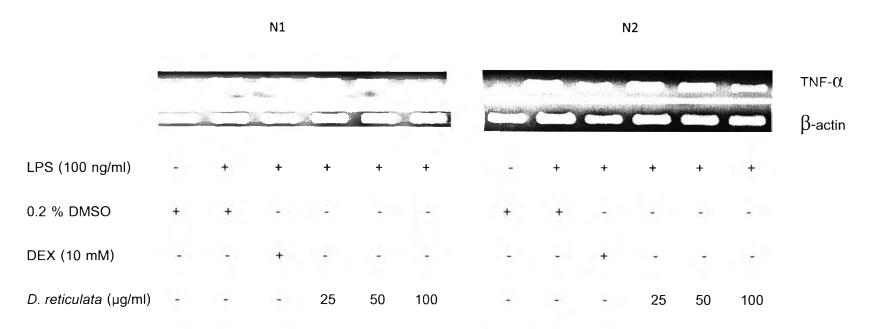


Figure 23: Effect of *D. reticulata* extract on the mRNA expression of TNF-α in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using TNF- α -specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

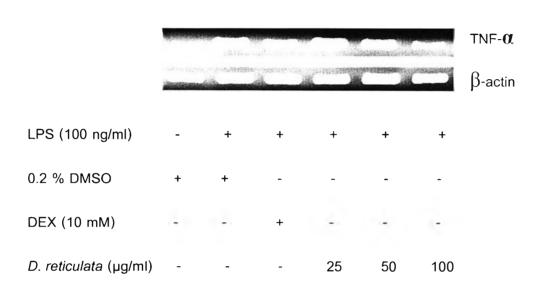


Figure 24: Effect of *D. reticulata* extract on the mRNA expression of TNF-α in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using TNF- α -specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

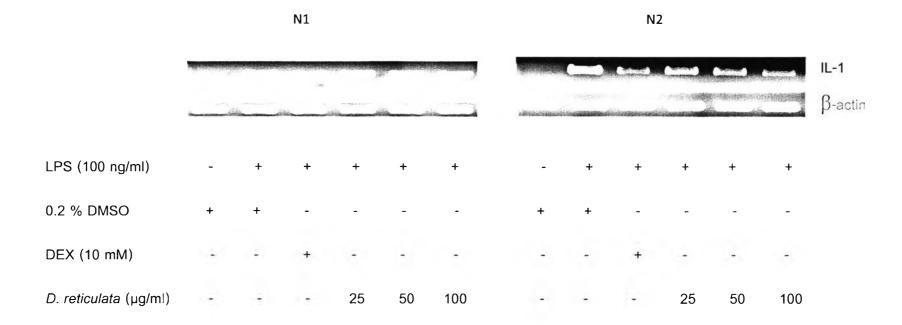


Figure 25: Effect of *D. reticulata* extract on the mRNA expression of IL-1 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using IL-1-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

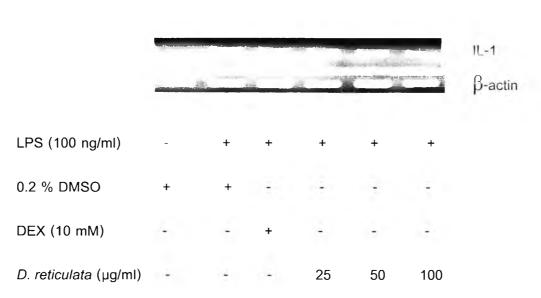


Figure 26: Effect of *D. reticulata* extract on the mRNA expression of IL-1 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using IL-1-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

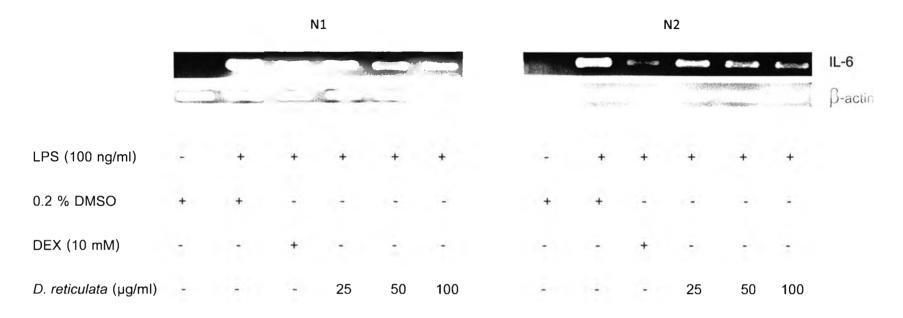


Figure 27: Effect of D. reticulata extract on the mRNA expression of IL-6 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using IL-6-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

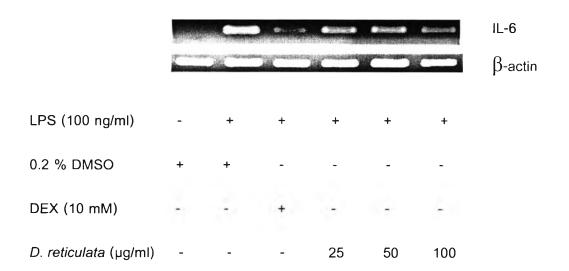


Figure 28: Effect of *D. reticulata* extract on the mRNA expression of IL-6 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using IL-6-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

Test compound		% of	control TI	NF-α	Mean ± S.E.	% of control IL-1			Mean ± S.E.	% of control IL-6			Mean ± S.E.
		1	2	3		1	2	3		1	1 2 3		1
0.2% DMSO		6.09	8.13	0.51	4.91±3.90	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00±0.00
LPS control		100.00	100.00	100.00	100.00±0.00	100.00	100.00	100.00	100.00±0.00	100.00	100.00	100.00	100.00±0.00
DEX (10μM)		50.93	48.52	47.83	49.09±1.60	69.41	69.25	68.84	69.17±0.30	30.11	30.85	28.96	29.97±0.95
	25	87.28	86.73	86.28	86.77±0.50	88.87	85.56	84.65	85.69±1.10	70.61	70.41	71.25	70.76±0.44
D. reticulata (µg/ml)·	50	62.78	61.20	60.87	61.62±1.02	77.69	77.97	77.46	77.71±0.26	63.82	62.97	63.71	63.50±0.45
-	100	39.81	39.21	38.67	39.23±0.57	53.73	53.87	51.32	52.97±1.43	39.72	37.00	39.15	38.63±1.43

Table 5: Data of the effect on the mRNA expression of pro-inflammatory cytokine (TNF- α , IL-1 and IL-6) of *D. reticulata* extract in LPS stimulated-J774A.1 cells.

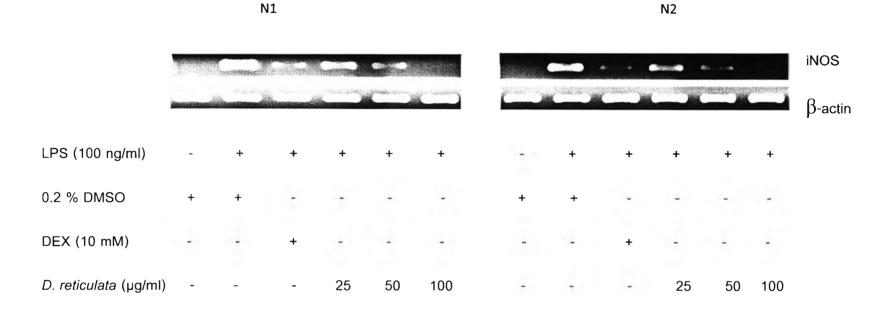


Figure 29: effect of *D. reticulata* extract on the mRNA expression of iNOS in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using iNOS-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

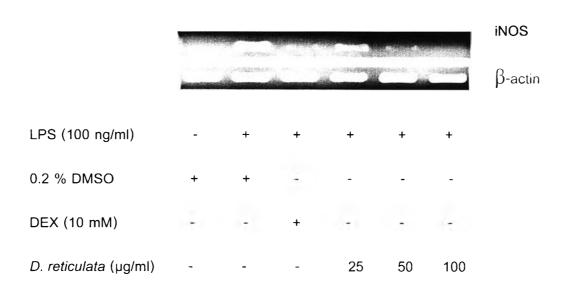


Figure 30: Effect of *D. reticulata* extract on the mRNA expression of iNOS in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using iNOS-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

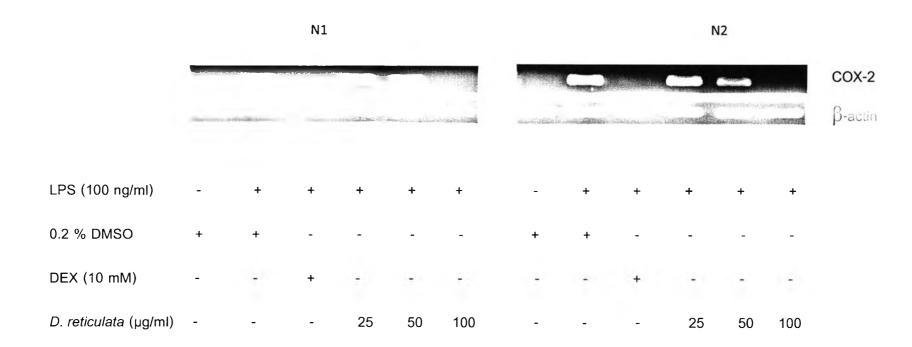


Figure 31: effect of *D. reticulata* extract on the mRNA expression of COX-2 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using COX-2-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.



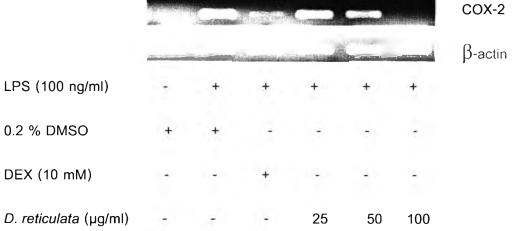


Figure 32: Effect of *D. reticulata* extract on the mRNA expression of COX-2 in LPS stimulated-J774A.1 cells.

J774A.1 cells were treated with 25, 50 and 100 μ g/ml for 24 h and then stimulated with 100 ng/ml LPS for 24 h. Total RNA was isolated from the treated cells and reverse transcribed to cDNA. The cDNA was amplified by PCR using COX-2-specific primer. The PCR products were run in 1.5% agarose gel electrophoresis and determine the quantities by using gel documentation and comparing with β -actin PCR product. Data are expressed as the mean \pm S.E., *p<0.05 indicates significant difference from LPS-stimulated control.

Teet U Wound		% C	of control in	10S	N ES.E.	% of	control Co	Maan ⊥ 1 E.	
		1	2	3		1	2	3	
0.2% DMSO		0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00±0.00
LPS control		100.00	100.00	100.00	100.00±0.00	100.00	100.00	100.00	100.00±0.0
DEX (10µM)		30.05	30.89	28.02	29.65±1.50	21.56	18.53	17.84	19.31±1.98
	25	51.06	48.93	51.49	50.49±1.40	94.89	96.40	94.91	95.40±0.87
D. reticulata (µg/ml)	50	38.93	39.65	37.64	38.74±1.02	70.15	69.93	67.13	69.07±1.70
100		11.62	10.10	9.85	10.52±0.96	12.84	12.51	11.17	12.17±0.88

Table 6: Data of the effect on the mRNA expression of iNOS and COX-2 of D. reticulata in LPS stimulated-J774A.1 cells.

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