

CHAPTER SEVEN

7. APPENDIX

7.1. Antioxidant study

7.1.1 Data of FRAP assay

Table 7.1: Data of standard curve of FRAP assay

Conc.	rep1	rep2	rep3	mean	avg-blk
0	0.091	0.09	0.09	0.090	
0	0.076	0.075	0.08	0.077	0.000
200	0.144	0.136	0.14	0.140	0.063
400	0.288	0.304	0.297	0.296	0.219
600	0.436	0.444	0.41	0.430	0.353
800	0.649	0.661	0.665	0.658	0.581
1000	0.709	0.714	0.723	0.715	0.638

STD: Ferrous Sulfate Hepta Hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)

Table 7.2: Antioxidant activity of *P. niruri* extract by FRAP assay

Treatment	rep1	rep2	rep3	mean	avg-blk	Frap value	SD	SEM
Ascorbic acid 10x	0.608	0.636	0.634	0.626	-12.374	-20623.3	0.016	0.012
quercetin 10x	0.371	0.357	0.363	0.364	-23.636	-39393.9	0.007	0.005
trolox 10x	0.219	0.213	0.217	0.216	-23.784	-39639.44	0.003	0.002
<i>P. niruri</i>	1.088	1.109	1.206	1.134	-22.866	-38109.44	0.063	0.048

*The initial concentration of samples were 0.001g/ml and final concentration were 33 μ g/cm³.

* Ascorbic acid, trolox & quercetin are diluted 10 times.

7.1.2 Data of DPPH assay

Table 7.3: Data of DPPH assay standard curve

Conc.	rep1	rep2	rep3	mean	% inhibition
0	0.43	0.437	0.43	0.432	0
5	0.391	0.383	0.369	0.381	11.87
10	0.338	0.322	0.316	0.325	24.75
15	0.29	0.261	0.264	0.272	37.16
20	0.251	0.193	0.223	0.222	48.57
25	0.208	0.165	0.16	0.178	58.91

Table 7.4: Antioxidant activity of *p.niruri* extract of DPPH assay

Treatment	rep1	rep2	rep3	average	%
<i>P.niruri</i> 50	0.095	0.094	0.094	0.094	66.47
<i>P.niruri</i> 25	0.183	0.177	0.169	0.176	37.32
<i>P.niruri</i> 12.5	0.229	0.237	0.253	0.240	14.81
<i>P.niruri</i> 6.25	0.266	0.279	0.285	0.277	1.66
<i>P.niruri</i> 3.125	0.291	0.295	0.288	0.291	-3.55
AC 50	0.075	0.078	0.074	0.076	73.10
AC 25	0.069	0.072	0.072	0.071	74.76
AC 12.5	0.074	0.077	0.075	0.075	73.22
AC 6.25	0.104	0.127	0.115	0.115	59.00
AC 3.125	0.246	0.241	0.255	0.247	12.09

IC₅₀ for *P.niruri* 37.61µg/Cm³

IC₅₀ for ascorbic acid 5.3 µg/ cm³

7.2 Experimental analysis

7.2.1 Counting the ACF

Table 6.5: Counting of ACF in tissues of rat's colon

groups	ACF counting in tissue					
	1 crypt	2	3	4	5 & more	Total
LD 1	3	7	13	24	22	69
LD 2	4	6	8	8	3	29
LD 3	5	12	8	5	5	35
LD 4	7	9	15	7	8	46
LD 5	6	6	10	2	2	26
Average LD	5	8	10.8	9.2	8	41
HD 1	1	6	12	6	8	33
HD 2	4	13	9	8	3	37
HD 3	6	8	4	7	16	41
HD 4	2	9	14	1	5	31
HD 5	2	4	1	1	2	10
Average HD	3	8	8	4.6	6.8	30.4
C5	10	19	23	15	24	91
C 2	0	8	14	10	9	41
C 4	8	26	22	23	38	117
C 7	49	59	47	24	18	197
C 6	29	23	15	13	12	92
Average C	14.6	18.2	18	11.4	14	107.6

LD: low, dose HD: high dose, C: control group Counting of ACF in colon tissue of the all groups.

7.4. Preparation of Reagents:

10% Tween - 20

100 ml of the stock solution was prepared by dissolving 10ml of 10% Tween-20 in 90 ml of distilled water.

0.9% Normal saline

9 g of NaCl was added to 1 L of distilled water.

(PBS) PH 7.3+ 0.2

1 Liter PBS was prepared as follows: sodium chloride 8.0 g L^{-1} , potassium chloride 0.2 g L^{-1} , di-sodium phosphate 1.15 g L^{-1} and potassium di-hydrogen phosphate 0.2 g L^{-1} were dissolved in distilled water at 25°C .

10% fresh formalin (Buffered formalin)

1 L of fresh formalin was prepared by dissolving 6.5 g di-sodium hydrogen phosphate with 4 g sodium di-hydrogen phosphate monohydrate and 100 ml concentrated formalin (38 - 40%) in 900 ml of phosphate buffer saline (PBS).