Table-1: Experimental schedule for *In-vitro* Splenocytes proliferation and IL-10 cytokine analysis.

Only spleen cells	Only spleen cells	Only spleen cells	Spleen cells + Con-A	Spleen cells + Con-A	Spleen cells + Con-A
Spleen cells + Extract 31.25µg/ml	Spleen cells + Extract 31.25µg/ml	Spleen cells + Extract 31.25µg/ml	Spleen cells + Extract 31.25µg/ml + Con-A	Spleen cells + Extract 31.25µg/ml + Con-A	Spleen cells + Extract 31.25µg/ml + Con-A
Spleen cells + Extract 62.5µg/ml	Spleen cells + Extract 62.5µg/ml	Spleen cells + Extract 62.5µg/ml	Spleen cells + Extract 62.5µg/ml + Con-A	Spleen cells + Extract 62.5µg/ml + Con-A	Spleen cells + Extract 62.5µg/ml + Con-A
Spleen cells + Extract 125µg/ml	Spleen cells + Extract 125µg/ml	Spleen cells + Extract 125µg/ml	Spleen cells + Extract 125µg/ml + Con-A	Spleen cells + Extract 125µg/ml + Con-A	Spleen cells + Extract 125µg/ml + Con-A
Spleen cells + Extract 250µg/ml	Spleen cells + Extract 250µg/ml	Spleen cells + Extract 250µg/ml	Spleen cells + Extract 250µg/ml + Con-A	Spleen cells + Extract 250µg/ml + Con-A	Spleen cells + Extract 250µg/ml + Con-A
Spleen cells + Extract 500µg/ml	Spleen cells + Extract 500µg/ml	Spleen cells + Extract 500µg/ml	Spleen cells + Extract 500µg/ml + Con-A	Spleen cells + Extract 500µg/ml + Con-A	Spleen cells + Extract 500µg/ml + Con-A

Table-2: ELISA Test Protocol

+ve control Sup.	+ve control Sup.	A1	A1	B1	B1	C1	C1	D1	D1	E1	E1
-ve control Sup.	-ve control Sup.	A2	A2	B2	B2	C2	C2	D2	D2	E2	E2
Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6
RB	RB										

+ve control Sup. = spleen cells supernatant + Con-A, -ve control Sup. = spleen cells supernatant only, RB = Reagent blank, A1-E1=500/250/125/62.5/31.25µg/ml extract and Con-A treated supernatant, respectively. A2-E2= 500/250/125/62.5/31.25µg /ml, extract treated supernatant, respectively. Std1-6= Rat IL-10 standard of concentration 1000/500/250/125/62.5/31.25 pg/ml, respectively.

***Table-3: In-vitro* antibacterial effects of HAE:**

S.No.	Name of Bacteria	Quantity of extract (mg/disc)	Zone of Inhibition (mm) after		
			24 hrs	36 hrs	48 hrs
1.	*Staph. aureus*	1.25	9	8	8
		2.5	10	9	9
		5	17	15	15
		10	20	19	19
		20	20	20	20
	Positive Control	Tetracycline (30µg)	30	30	30
2.	*Bacillus cerus*	1.25	8	8	7
		2.5	12	10	9
		5	15	15	14
		10	18	17	17
		20	19	19	18
	Positive Control	Tetracycline (30µg)	23	23	23
3.	*Streptococcus uberis*	1.25	9	9	9
		2.5	12	12	12
		5	16	16	16
		10	19	19	18
		20	22	22	22
	Positive Control	Tetracycline (30µg)	27	27	27
4.	*E. coli*	1.25	0	0	0
		2.5	8	8	7
		5	9	8	8
		10	11	10	10
		20	15	14	14
	Positive Control	Amikacin (30 µg)	24	24	24
5.	*Pseudomonas aeruginosa*	1.25	8	8	7
		2.5	10	9	8
		5	13	12	12
		10	15	13	13
		20	15	13	12
	Positive Control	Amikacin (30 µg)	18	18	18
6.	*Klebsiella pneumoniae*	1.25	9	9	8
		2.5	10	9	8
		5	13	12	12
		10	13	13	13
		20	13	13	13
	Positive Control	Amikacin (30 µg)	25	25	25
7.	Negative control	0	–	–	–

Table-4: ***In-vitro*** **antifungal effects of HAE:**

S.No.	Name of Fungus	Quantity of extract (mg/disc)	Zone of Inhibition (mm) after		
			24 hrs	36 hrs	48 hrs
1.	***Candida albicans***	2.5	–	–	–
		5	–	–	–
		10	10	–	–
		20	11	–	–
		Fluconazole (10µg)	28	28	28
2.	***Aspergillus niger***	2.5	–	–	–
		5	8	–	–
		10	8	8	7
		20	12	11	11
		Fluconazole (10µg)	21	21	21
3.	***Aspergillus fumigates***	2.5	–	–	–
		5	8	–	–
		10	9	8	8
		20	13	13	12
		Fluconazole (10µg)	23	23	23
4.	Negative control	0	–	–	–

Table-5: MNTD of HAE of *Acacia nilotica* leaves in MDBK cell line

S.No.	Conc. of HAE of *A. nilotica* (mg/ml)	Absorbance (O.D)* (Mean ± S.E.)	Control (Mean ± S.E.)
1.	0.3125	1.579 ± 0.083	1.190 ± 0.046
2.	0.625	1.471 ± 0.028	
3.	1.25	1.458 ± 0.228	
4.	2.5	1.076 ± 0.012	
5.	5	0.905 ± 0.125	
6.	10	0.812 ± 0.091	

Difference (O.D. at 560 - 670 nm)*

Table-06: $TCID_{50}$ for IBR virus

S.N.	Log of virus concentration	Optical density* (Mean ± S.E.)
1	0	0.400 ± 0.031
2	-1	0.757 ± 0.049
3	-2	1.102 ± 0.048
4	-3	1.437 ± 0.070
5	-4	1.491 ± 0.022
6	-5	1.561 ± 0.013
7	-6	1.586 ± 0.051
8	-7	1.587 ± 0.025
9	-8	1.561 ± 0.051
10	-9	1.553 ± 0.044
11	-10	1.61 ± 0.025
12	Control	1.546 ± 0.046
Difference (O.D. at 560 - 670 nm)*		

Table-07: Antiviral effect of HAE of *Acacia nilotica* leaves against IBR virus

S.N.		Absorbance (O.D.)* (Mean ± S.E.)		
		0.3125mg/ml	0.625mg/ml	1.25mg/ml
1	HAE of *A. nilotica* + $TCID_{50}$ IBR	0.413 ± 0.037	0.414 ± .052	0.433 ± .028
2	Virus control	0.710 ± 0.020		
3	Cell control	0.992 ± 0.013		
4.	Percentage protection	Not Detectable		

Difference (O.D. at 560 - 670 nm)*

Table-08: *In-vitro* effect of HAE of *Acacia nilotica* leaves on Splenocyte proliferation in Wistar albino rats

S.No.	Conc. of extract (µg/ml)	Optical densities* (Mean ± S.E.)		% Proliferation/inhibition	
		Without Con-A	With Con-A	Without Con-A	With Con-A
1	Control**	0.208 ± 0.006	0.403 ± 0.012		
2	31.25	0.246 ± 0.005	0.448 ± 0.004	18.27	11.17
3	62.5	0.267 ± 0.002	0.459 ± 0.004	28.36	13.18
4	125	0.253 ± 0.004	0.313 ± 0.009	21.63	-22.33
5	250	0.228 ± 0.001	0.255 ± 0.015	9.61	-36.72
6	500	0.113 ± 0.006	0.120 ± 0.003	-45.67	-70.22

Difference (O.D. at 560 - 670 nm)*

Control** without Con-A = -ve Control = only spleen cells.

Control** with Con-A = +ve Control = Spleen cells + Con-A.

Table-09: *In-vitro* effect of HAE of *Acacia nilotica* leaves on Cytokine IL-10 induction

S.No.	Conc. of extracts (µg/ml)	Optical densities* (Mean ± S.E.)		% Change	
		Without Con-A	With Con-A	Without Con-A	With Con-A
1	Control**	1.239 ± 0.014	1.531 ± 0.023		
2	31.25	0.932 ± 0.082	1.319 ± 0.042	-24.78	-13.85
3	62.5	1.162 ± 0.000	1.494 ± 0.000	-6.21	-2.42
4	125	1.156 ± 0.002	1.430 ± 0.036	-6.69	-6.59
5	250	1.138 ± 0.002	1.366 ± 0.000	-8.15	-10.78
6	500	1.092 ± 0.006	1.208 ± 0.001	-11.86	-21.09

Control** without Con-A = -ve Control = only spleen cells.

Control** with Con-A = +ve Control = Spleen cells + Con-A.

Optical densities* (absorbance at 450nm)