

The Effect of Wheatgrass Lyophilizate on Blood Clotting Time in Rats

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Supplemental material content:

Supplementary Table S1. Freeze-drying process

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Supplementary Table S3–S7. Weight of animals, amount of administered warfarin and wheatgrass on 1–5 day of treatment.

Supplementary Figure S1. HPLC chromatogram of reference compounds ($\lambda=350$ nm)

Supplementary Figure S2. HPLC chromatogram of fraction eluted with 40% of methanol and apigenin ($\lambda=350$ nm)

Supplementary Figure S2. Calibration curve (apigenin).

Table S1. Freeze-drying process.

Process	Temperature (°C)	Interval (hours)	Pressure (Mbar)
Sublimation	−54	5.0	0.15
	−40	2.5	0.15
	−30	2.0	0.25
	−10	2.0	0.30
Drying	−5	5.0	0.30
	+10	4.0	0.40
Secondary drying	+30	5.0	0.50

Table 2. Database of prothrombin times before and after treatment used for statistical analysis.

Group	Animal #	mechanical coagulometer		optical coagulometer	
		before	after	before	after
WG	1	17.4	16.7	20.9	18.3
WG	1	17.3	16.7	21.3	18.1
WG	1	17.5	16.8	-	-
WG	2	15.3	16.4	20.7	18.3
WG	2	15.2	17	20.7	18.5
WG	2	15.4	17	19.7	18.7
WG	3	15	17.9	18.3	18.3
WG	3	15	17.1	18.1	17.9
WG	3	15	17.2	-	-
WG	4	16	20.3	18	19.7
WG	4	16	18.7	17.7	19.9
WG	4	15.9	18.5	-	-
WG	5	16.4	18	18.7	17.9
WG	5	16.4	17	18.5	17.9
WG	5	16.4	17.1	-	-
WG	6	17	20	18.9	20.3
WG	6	16.9	18.8	18.9	20.3
WG	6	17.1	18.4	-	-
WG	7	17	18.1	-	-
WG	7	16.9	16.1	-	-
WG	7	17.1	15.1	-	-
WG	8	17.6	20.7	19.8	20.5
WG	8	17.7	19.5	24.9	20.2
WG	8	17.5	19.2	-	-
WF	9	16.5	40	17.7	40.1
WF	9	16.5	36.2	17.5	30.1
WF	9	16.5	37.8	-	-
WF	10	17.2	35.5	18.3	35.7
WF	10	17.3	33.1	18.3	35.9
WF	10	17.1	30	-	-
WF	11	18.1	39.9	19.3	40.7
WF	11	18.2	36.1	19.7	40.1
WF	11	18	35.9	-	-
WF	12	17.1	39.5	17.7	38.8
WF	12	17.1	40.5	18.1	38.3
WF	12	17.1	35.5	18.1	41.7
WF	14	18.3	41.2	18.9	39.5
WF	14	18.4	39.3	18.7	40.7
WF	14	-	-	-	-
WF	16	17.5	35.9	18.9	34.7
WF	16	17.6	35.1	18.3	34.9
WF	16	17.4	35.3	-	-
WF	17	19.2	26.4	-	-
WF	17	19.2	26.4	-	-
WF	17	-	-	-	-
WW	18	17.6	46.7	18.7	46.1
WW	18	17.7	36.8	18.7	47.3
WW	18	17.5	39.3	-	-

WW	19	18	34.7	18.7	35.5
WW	19	18.1	32.1	18.7	35.5
WW	19	17.9	32.1	-	-
WW	22	17.2	50.8	17.9	50.1
WW	22	17.2	45.9	17.9	50.3
WW	22	17.2	46.7	-	-
WW	23	16.3	43.5	16.9	44.7
WW	23	16.2	39.8	16.7	43.7
WW	23	16.1	39.5	-	-
WW	24	17.3	49.1	18.3	47.9
WW	24	17.3	44	17.9	48.7
WW	24	17.3	44	-	-

WG: wheatgrass; WF: warfarin; WW: wheatgrass+warfarin; -: determination of prothrombin time failed

Table S3. 1st day of treatment

Group	Animal	Weight of the animal (g)	Ammount of warfarin (mg)	Ammount of tablet (mg)	Ammount of wheatgrass (mg)
WG	1.	245			31.24
	2.	244			31.11
	3.	240			30.60
	4.	257			32.77
	5.	247			31.49
	6.	244			31.11
	7.	254			32.39
	8.	237			30.22
WF	9.	261	0.1044	4.78	
	10.	262	0.1048	4.80	
	11.	250	0.1000	4.58	
	12.	259	0.1036	4.75	
	13.	247	0.0988	4.53	
	14.	240	0.0960	4.40	
	15.	231	0.0924	4.23	
	16.	238	0.0952	4.36	
WW	17.	252	0.1008	4.62	32.13
	18.	232	0.0928	4.25	29.58
	19.	247	0.0988	4.53	31.49
	20.	233	0.0932	4.27	29.71
	21.	251	0.1004	4.60	32.00
	22.	252	0.1008	4.62	32.13
	23.	271	0.1084	4.97	34.55
	24.	275	0.1100	5.04	35.06

Further notes:

Weight of warfarin: 137.42 mg

Observations:

The animal No 17. is eating slow.

The doughs had to be put in the paws for several times in case of animal No 23. and 24., but finally the feeding was successful.

The WW group received warfarin and wheatgrass in the same dough.

Table S4. 2nd day of treatment

Group	Animal	Weight of the animal (g)	Ammount of warfarin (mg)	Ammount of tablet (mg)	Ammount of wheatgrass (mg)
WG	1.	249			31.75
	2.	248			31.62
	3.	244			31.11
	4.	260			33.15
	5.	249			31.75
	6.	248			31.62
	7.	256			32.64
	8.	240			30.60
WF	9.	264	0.1056	4.82	
	10.	264	0.1056	4.82	
	11.	251	0.1004	4.59	
	12.	260	0.1040	4.75	
	13.	247	0.0988	4.51	
	14.	240	0.0960	4.38	
	15.	237	0.0948	4.33	
	16.	246	0.0984	4.49	
WW	17.	256	0.1024	4.68	32.64

18.	236	0.0944	4.31	30.09
19.	252	0.1008	4.60	32.13
20.	231	0.0924	4.22	29.45
21.	256	0.1024	4.68	32.64
22.	251	0.1004	4.59	32.00
23.	278	0.1112	5.08	35.45
24.	278	0.1112	5.08	35.45

Further notes:

Weight of warfarin: 137.02 mg

Observations:

WW group received warfarin and wheatgrass in separated dough.

Table S5. 3rd day of treatment

Group	Animal	Weight of the animal (g)	Ammount of warfarin (mg)	Ammount of tablet (mg)	Ammount of wheatgrass (mg)
WG	1.	257			32.77
	2.	256			32.64
	3.	248			31.62
	4.	269			34.30
	5.	256			32.64
	6.	251			32.00
	7.	260			33.15
	8.	245			31.24
WF	9.	269	0.1076	5.07	
	10.	269	0.1076	5.07	
	11.	247	0.0988	4.66	
	12.	269	0.1076	5.07	

WW	13.	255	0.1020	4.81	
	14.	246	0.0984	4.64	
	15.	237	0.0948	4.47	
	16.	249	0.0996	4.70	
	17.	258	0.1032	4.87	32.90
	18.	242	0.0968	4.56	30.86
	19.	252	0.1008	4.75	32.13
	20.	238	0.0952	4.49	30.35
	21.	262	0.1048	4.94	33.41
	22.	260	0.1040	4.90	33.15
	23.	289	0.1156	5.45	36.85
	24.	290	0.1160	5.47	36.98

Further notes:

Weight of warfarin: 141.45 mg

Observations: ---**Table S6.** 4th day of treatment

Group	Animal	Weight of the animal (g)	Amount of warfarin (mg)	Amount of tablet (mg)	Amount of wheatgrass (mg)
WG	1.	262			33.41
	2.	258			32.90
	3.	256			32.64
	4.	273			34.81
	5.	260			33.15
	6.	258			32.90
	7.	259			33.02
	8.	245			31.24

WF	9.	271	0.1084	5.15	
	10.	269	0.1076	5.11	
	11.	257	0.1028	4.89	
	12.	270	0.1080	5.13	
	13.	257	0.1028	4.89	
	14.	245	0.0980	4.66	
	15.	237	0.0948	4.51	
	16.	249	0.0996	4.73	
WW	17.	263	0.1052	5.00	33.53
	18.	242	0.0968	4.60	30.86
	19.	261	0.1044	4.96	33.28
	20.	244	0.0976	4.64	31.11
	21.	270	0.1080	5.13	34.43
	22.	267	0.1068	5.08	34.04
	23.	285	0.1140	5.42	36.34
	24.	290	0.1160	5.51	36.98

Further notes:

Weight of warfarin: 142.61 mg

Observations: ---**Table S7.** 5th day of treatment

Group	Animal	Weight of the animal (g)	Ammount of warfarin (mg)	Ammount of tablet (mg)	Ammount of wheatgrass (mg)
WG	1.	265			33.79
	2.	269			34.30
	3.	261			33.28

WF	4.	276			35.19
	5.	262			33.41
	6.	261			33.28
	7.	261			33.28
	8.	249			31.75
	9.	279	0.1116	5.14	
	10.	272	0.1088	5.02	
	11.	262	0.1048	4.83	
	12.	279	0.1116	5.14	
	13.	267	0.1068	4.92	
WW	14.	252	0.1008	4.65	
	15.	245	0.0980	4.52	
	16.	255	0.1020	4.70	
	17.	275	0.1100	5.07	35.06
	18.	248	0.0992	4.57	31.62
	19.	263	0.1052	4.85	33.53
	20.	246	0.0984	4.54	31.37
	21.	275	0.1100	5.07	35.06
	22.	267	0.1068	4.92	34.04
	23.	299	0.1196	5.51	38.12
	24.	303	0.1212	5.59	38.63

Further notes:

Weight of warfarin: 138.3 mg

Observations: ---

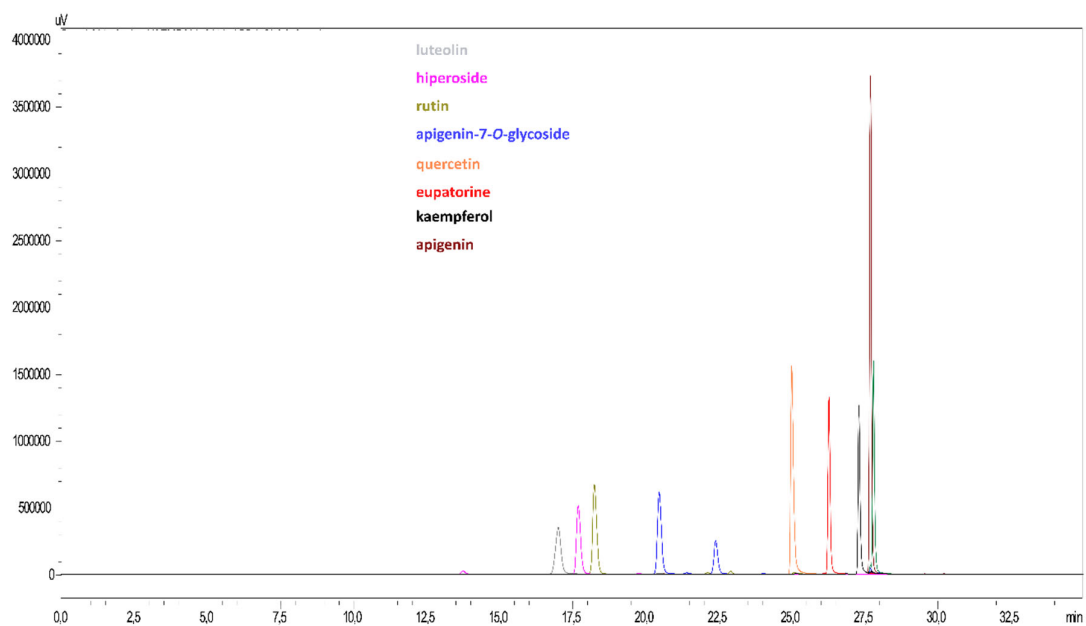


Figure S1. HPLC chromatogram of reference compounds ($\lambda=350$ nm).

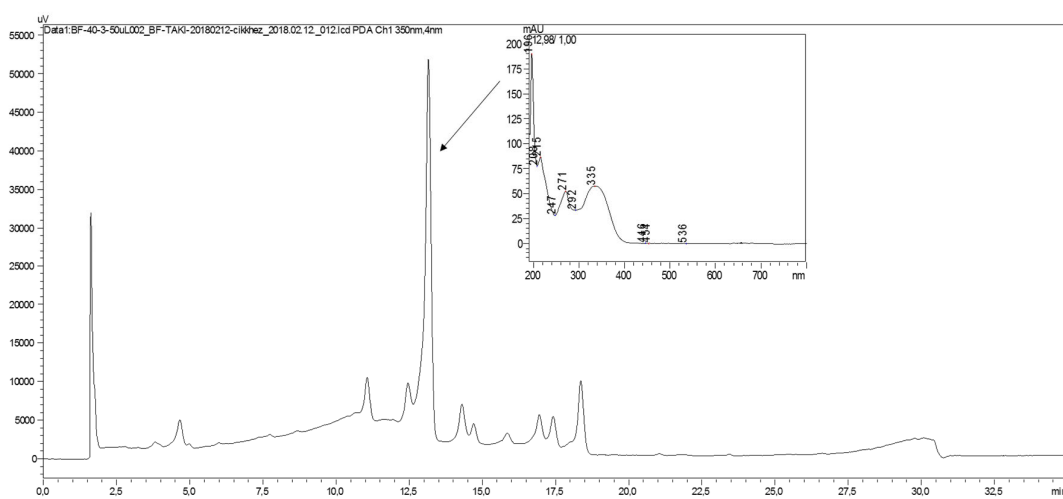


Figure S2. HPLC chromatogram of fraction eluted with 40% of methanol ($\lambda=350$ nm) and the UV spectrum of the major peak at $R_t = 13$ min.

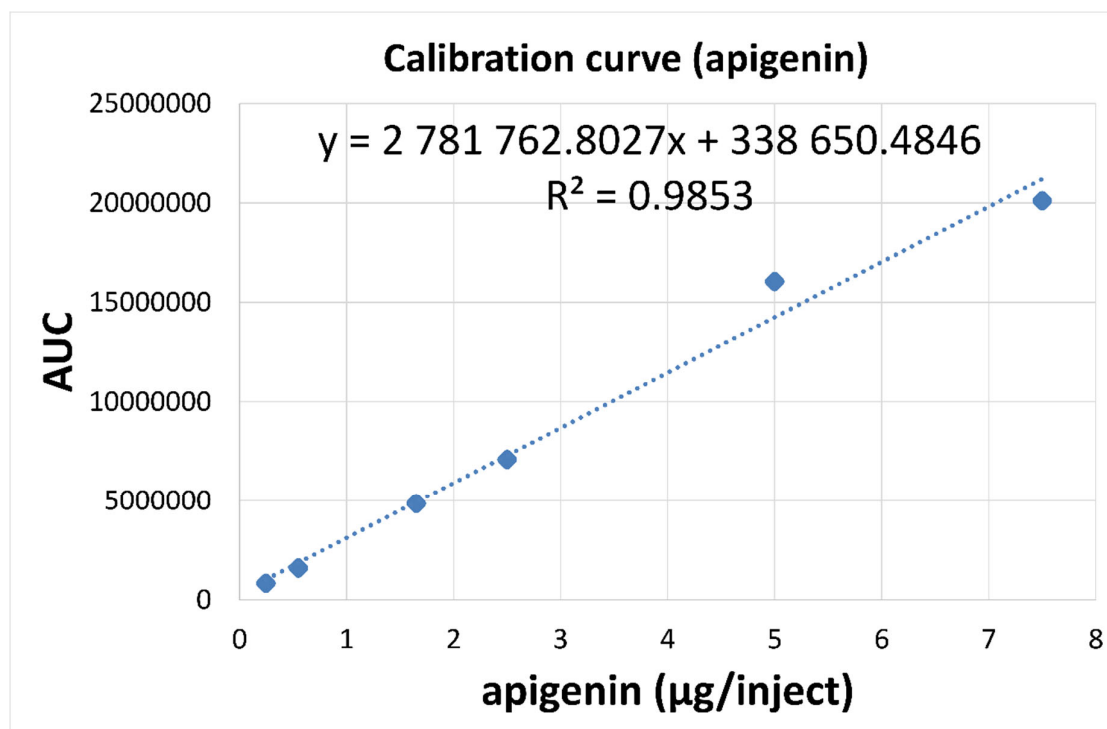


Figure S3. Calibration curve (apigenin).