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Research Article

A propsective oncotherapeutics: Isolation, partial purification and assessment of anti-coagulant properties of *Tridax procumbens* from leaf extracts

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Supplementary Tab. 1. Method conditions for the purification of the crude leaf extract.

S.No	Phase	Buffer	CV	Flow mL/min	Remarks
1	Equilibration	20 mM Tris (pH=8.0)	3	10	Fast equilibration
2	Equilibration	20 mM Tris (pH=8.0)	2	6	Slow equilibration
3	Injection	10 mL of extract as a sample	NA	5	Crude extracts
5	Column wash	20 mM Tris+10mM NaCl (pH=8.0)	2	4	Buffer+10mM NaCl
6	Elution 1	20 mM Tris+0.01 to 2.5 NaCl (pH=8.0)	2	4	Linear gradient
7	Elution 2	20 mM Tris+2.5 NaCl (pH=8.0)	0.1	4	Step gradient
8	Column wash	20 mM Tris (pH=8.0)	3	8	20 mM Tris(pH=8.0)
9	Regeneration	20 mM Tris+2.5 NaCl (pH=8.0)	1	8	20 mM Tris+2.5 NaCl (pH=8.0)

Supplementary Tab. 2. Method conditions for the purification of the crude leaf extract.

Tube No.	Vol. of Saline (mL)	Vol. of working dilution of Heparin (1 IU/mL)* standard or test sample solution (mL)	Plasma (mL)	1% CaCl2 solution (mL)	Total volume (mL)
1	0.4	0.4	1	0.2	2
2	0.38	0.42	1	0.2	2
3	0.36	0.44	1	0.2	2
4	0.34	0.46	1	0.2	2
5	0.32	0.48	1	0.2	2
6	0.3	0.5	1	0.2	2
7	0.28	0.52	1	0.2	2
8	0.25	0.55	1	0.2	2
9	0.22	0.58	1	0.2	2
10	0.19	0.61	1	0.2	2
11	0.16	0.64	1	0.2	2
12	0.13	0.67	1	0.2	2
13	0.09	0.71	1	0.2	2
14	0.05	0.75	1	0.2	2
15	0	0.8	1	0.2	2

Supplementary Tab. 3. Reagent dilution for anti-factor Xa.

Reagents for anti-FXa assay	Starting material		Volume of buffer	Final conc.
	Stock conc.	Volume		
ATIII	5 IU/mL	1000 µL	4000 µL of pH 7.4 PEG buffer	1 IU/mL
Factor Xa	7.1 nkats/mL	3000 µL	3000 µL of pH 7.4 PEG buffer	3.55 nkats/mL
Chromogenic substrate for FXa	3mM	1500 µL	7500 µL of pH 8.4 buffer	0.5 mM

Supplementary Tab. 4. Reagent dilution for anti-factor Xa.

Reagents for anti-FIIa assay	Starting Material		Volume of diluent	Final conc.
	Stock conc.	Volume		
ATIII	5 IU/mL	500 µL	4500 µL of pH 7.4 PEG buffer	0.5 IU/mL
Factor IIa	100 NIH/mL	180 µL	5820 µL of pH 7.4 PEG buffer	3 NIH/mL
Chromogenic substrate for FIIa	3 mM	1500 µL	7500 µL of pH 8.4 buffer	0.5 mM