Facile purification of milligram to gram quantities of condensed tannins according to mean degree of polymerization and flavan-3-ol subunit composition

Article

Supplemental Material

Supplementary Materials for:

Facile Purification of Milligram to Gram Quantities of Condensed Tannins According to Mean Degree of Polymerization and Flavan-3-ol Subunit Composition

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Methods and Materials:

1. Specific Example for Preparation of a Crude Plant Extract.

Finely ground sericea lespedeza (50.1 g) was transferred to a 500 mL Erlenmeyer flask equipped with a magnetic stir bar and a mixture of 7:3 acetone/water (300 mL) was added. The mixture was rapidly stirred for 30 min and then filtered through a 600 mL Buchner funnel equipped with a filter paper. The retained solids were subjected to this extraction process two additional times and the combined filtrates were concentrated under reduced pressure on a rotary evaporator (< 35 ºC) to remove acetone. Dichloromethane (200 mL) was added to the resulting aqueous layer and gently stirred (to avoid formation of an emulsion) using a magnetic stir bar (30 min). The layers were transferred to a separatory funnel and the aqueous layer was re-subjected to dichloromethane extraction two additional times (2 x 150 mL). The aqueous layer was concentrated under reduced pressure on a rotary evaporator (<35 °C) to remove traces of dichloromethane and then freeze-dried to give 10.7 g of a tan solid and labeled as **crude extract**.

2. Detailed Description of 'Wide column' Sephadex LH-20 Chromatography (*Method 3*).

1. Sephadex LH-20 (50 g) was left to swell in water (200 mL) for at least 4 hours.
2. The resulting slurry was then poured into a glass column (400 mm length x 65 mm i.d.) equipped with a sintered-glass frit followed by water (1 L).
3. The slurry was allowed to settle and the excess water was drained to a level of 10 mm above the resulting resin bed (70 mm length x 65 mm i.d.).
4. Plant samples (50 g) were extracted with acetone/water (7:3, v/v; 500 mL) by stirring at r.t. for 40 min. The extract was filtered, extracted in a separatory funnel with dichloromethane (250 mL), the upper, aqueous phase concentrated on a rotary evaporator to remove acetone and centrifuged to remove any insoluble material.
5. The aqueous phase containing ca 10 g of this crude plant extract was stored in the freezer overnight and thawed the next day, diluted with deionized water (1 L), filtered and water (1 L) added.
6. This solution was transferred to a separatory funnel, which was placed above the glass column so that the funnel outlet touched the side of the glass column.
7. The funnel stopcock was opened and the CT solution allowed to flow, slowly initially, along the inside of the column without disturbing the resin bed, and then rapidly once sufficient liquid was above the column, onto the Sephadex LH-20 resin.
8. The column stopcock was opened to give a fast flow (ca 40 mL/min) through the column and sample application was stopped when the solvent reached ca 10 mm above the resin.
9. Then 2 L of deionized water was added to the funnel and the column was rinsed until the eluent was clear (flow rate: 40 to 50 mL/min) and rinsing was stopped when the water level was 10 mm above the resin.
10. Acetone/water (3:7, v/v, 1 L) was then added to the separatory funnel and the first 200 mL of eluent were discarded.
11. The CTs were collected at 15 mL/min during the next 500 mL elution giving **Fraction 1** (vanillin/HCl was used to test for CTs in eluent).[1]
12. The remaining solvent was used to rinse the column and the flow was stopped again when the solvent reached 10 mm above the resin.
13. **Fraction 2** was similarly eluted with acetone/water (1:1, v/v, 1 L), where the first 200 mL of eluent was discarded and CTs were collected at 25 mL/min during the next 300 mL of elution.
14. The column was reconditioned with water (2 L) at 25 mL/min.
15. Columns could be re-used approximately 10 times without losing separation efficiency as monitored by thiolysis of each fraction. Occasional rinsing of the resin with acetone/water (8:2, v/v) extended column life when necessary.

1. Schofield, P.; Mbugua, D. M.; Pell, A. N. 2001. Analysis of condensed tannins; a review. *Anim. Feed Technol.* **2001**, *91*, 21-40.

3. Repurification of a Sericea Lespedeza CT Fraction for Demonstration of ^{1}H NMR Spectroscopy as a CT Purity Screening Tool.

An impure fraction from a previous *Lespedeza cuneata* CT purification attempt (1.2 g, fraction **F2**) was dissolved in methanol/water (1:1, v/v; 60 mL) and Sephadex LH-20 resin was added in small portions with stirring until a thick slurry developed with the consistency of wet sand (a total of 13.1 g Sephadex LH-20 was added). The resin was transferred to a 600 mL coarse sintered-glass funnel equipped with a filter paper, suspended in the solvent (listed below), allowed to stand for 5-10 min and then vacuum-filtered using the following sequence of solvents: methanol/water (1:1, v/v; 5 x 50 mL) to give fraction **P2F0**; acetone/water (1:1, v/v; 5 x 50 mL) to give fraction **P2F2**; acetone/water (7:3, v/v; 5 x 50 mL) to give fraction **P2F3**. Fractions were concentrated <35 ºC to remove the acetone and the resulting aqueous phases freeze-dried (Yields: fraction **P2F0**, 435 mg; fraction **P2F2**, 524 mg; fraction **P2F3**, 99 mg).

Table S1. Applied and recovered masses from chromatography of a crude sainfoin extract (Cotswold Common; 379.5 mg) on standard columns (120 mm length x 30 mm i.d.) of either Sephadex LH-20 or Toyopearl HW-50F (*Method 2*).

Columns and eluents	**Fraction**	**Weight (mg)**	**Percentage of applied sample (%)**	**Percentage of recovered extract %**	**Sum per eluent %**
Sephadex LH-20					
Water	S1	40.4	10.65	11.4	
	S2	4.8	1.27	1.4	
	S3	2.6	0.69	0.7	**13.5**
Methanol/water (1:1, v/v)	S4	4.8	1.27	1.4	
	S5	13.6	3.59	3.8	
	S6	10.9	2.87	3.1	**8.3**
Acetone/water (7:3, v/v)	S7	118.7	31.29	33.4	
	S8a	105.5	27.81	29.7	
	S8b	32.6	8.59	9.2	
	S9	18	4.75	5.1	**77.4**
Acetone	S10	3.2	0.84	0.9	**1.1**
Recovery		**355.1**	**93.62**	**100.0**	
Toyopearl HW-50F					
Water	T1	28.0	7.38	10.4	
	T2	4.5	1.19	1.7	
	T3	1.5	0.40	0.6	**12.7**
Methanol/water (1:1, v/v)	T4	2.5	0.66	0.9	
	T5	4.9	1.29	1.8	
	T6	6.1	1.61	2.3	**5.0**
Acetone/water (7:3, v/v)	T7	80	21.09	29.8	
	T8a	83.9	22.12	31.3	
	T8b	16.2	4.27	6.0	
	T9	37.5	9.89	14.0	**81.2**
Acetone	T10	3	0.79	1.1	**1.1**
Recovery		**268.1**	**70.68**	**100.0**	

Table S2. Flavan-3-ol subunit composition (molar percentages) of condensed tannins from sainfoin (Cotswold Common) separated on either Sephadex LH-20 or Toyopearl HW-50F columns (*Method 2*).

Columns and eluents	Fraction	Terminal units (%)				Extension units (%)			
Sephadex LH-20		GC	EGC	C	EC	GC	EGC	C	EC
Water	S1	-	-	3.3 (0.4)	4.9 (0.5)	-	21.5 (1.5)	-	70.3 (1.5)
	S2	-	-	5.7 (1.0)	16.3 (0.3)	-	18.9 (1.8)	-	59.1 (1.7)
	S3	-	-	4.5 (0.4)	17.4 (0.9)	-	17.6 (3.0)	-	60.5 (3.1)
Methanol/water (1:1, v/v)	S4	-	-	4.2 (0.6)	13.6 (0.3)	-	19.3 (0.1)		62.9 (0.8)
	S5	-	-	5.5 (0.3)	11.2 (0.8)	-	24.8 (3.3)	-	58.6 (2.6)
	S6	-	-	4.9 (1.9)	9.0 (0.2)	-	20.3 (2.9)	-	65.9 (1.5)
Acetone/water (7:3, v/v)	S7	1.1 (0.1)	1.6 (0.1)	3.2 (0.1)	3.9 (0.1)	7.0 (0.3)	42.4 (0.2)	3.2 (0.1)	37.6 (0.3)
	S8a	1.9 (0.1)	2.4 (0.1)	4.4 (0.0)	5.1 (0.0)	9.0 (0.0)	35.6 (0.1)	5.3 (0.1)	36.4 (0.2)
	S8b	2.2 (0.1)	2.6 (0.1)	3.7 (0.0)	4.1 (0.1)	10.4 (0.3)	41.1 (0.3)	4.1 (0.1)	31.8 (0.4)
	S9	2.0 (0.2)	2.7 (0.4)	2.7 (0.1)	2.7 (0.1)	10.5 (0.3)	48.1 (0.4)	3.1 (0.2)	28.0 (0.8)
Acetone	S10	1.3 (0.2)	1.8 (0.1)	1.8 (0.2)	1.9 (0.1)	11.2 (0.2)	51.6 (0.3)	3.0 (0.1)	27.4 (0.3)
Toyopearl HW-50F									
Water	T1	-	-	1.2 (0.2)	1.5 (0.1)	5.7 (0.7)	61.5 (0.8)	1.9 (0.2)	28.2 (0.2)
	T2	-	-	-	-	-	-	-	100 (0.0)
	T3	-	-	-	-	-	-	-	-
Methanol/water (1:1, v/v)	T4	-	-	-	-	-	-	-	-
	T5	-	-	8.5 (0.8)	12.0 (0.9)	-	10.4 (4.3)	6.6 (0.8)	62.5 (1.9)
	T6	-	-	-	10.9 (2.8)	-	-	6.3 (0.3)	82.8 (3.0)
Acetone/water (7:3, v/v)	T7	1.5 (0.1)	1.7 (0.1)	4.5 (0.1)	5.1 (0.0)	5.3 (0.3)	33.7 (0.1)	4.1 (0.1)	44.2 (0.2)
	T8a	1.7 (0.2)	2.1 (0.2)	3.2 (0.1)	3.9 (0.1)	9.6 (0.1)	44.8 (0.4)	4.1 (0.1)	30.7 (0.1)
	T8b	1.0 (0.0)	1.3 (0.2)	0.9 (0.0)	1.2 (0.0)	11.2 (0.3)	70.7 (0.3)	1.5 (0.1)	12.3 (0.1)
	T9	0.5 (0.1)	0.4 (0.1)	0.5 (0.0)	0.5 (0.0)	9.8 (0.4)	77.9 (0.5)	0.9 (0.1)	9.4 (0.2)
Acetone	T10	0.5 (0.1)	0.5 (0.1)	0.6 (0.0)	0.7 (0.1)	9.2 (0.2)	75.2 (0.1)	1.3 (0.0)	12.1 (0.1)

*) -: none detected

GC = gallocatechin, EGC = epigallocatechin, C = catechin, EC = epicatechin

Table S3. Flavan-3-ol subunit compositions (molar percentages) of condensed tannin fractions obtained by wide Sephadex LH-20 column chromatography (*Method 3*). (n=1 to 9 separate fractionations). Fractions were analyzed by thiolytic degradation.

	Terminal units (%)				**Extension units (%)**			
	GC	**EGC**	**C**	**EC**	**GC**	**EGC**	**C**	**EC**
Birdsfoot trefoil								
F1 (n=5)	-	-	19.6 (2.42)	6.8 (1.08)	-	16.8 (0.75)	3.6 (0.21)	53.2 (2.97)
F2 (n=4)	-	-	5.9 (0.93)	2.1 (0.36)	-	33.0 (2.46)	3.0 (0.13)	56.0 (1.14)
F3 (n=3)	-	-	2.5 (0.61)	0.8 (0.22)	-	26.2 (8.37)	2.0 (0.81)	68.4 (9.10)
Big trefoil								
F1 (n=4)	-	-	12.6 (0.94)	6.0 (0.47)	15.7 (2.74)	49.0 (3.24)	4.4 (0.31)	12.3 (0.52)
F2 (n=4)	-	-	4.0 (0.23)	1.6 (0.11)	8.5 (0.08)	66.6 (0.55)	3.0 (0.06)	16.3 (0.22)
Sericea lespedeza								
F1 (n=3)*	13.1 (0.06)	5.3 (0.01)	1.0 (0.00)	1.0 (0.03)	18.9 (0.06)	55.2 (0.07)	1.7 (0.02)	3.9 (0.05)
F2 (n=3)*	5.8 (0.18)	2.1 (0.00)	0.4 (0.00)	0.6 (0.20)	17.0 (0.06)	67.5 (0.39)	1.6 (0.02)	5.1 (0.03)
F3 (n=3)*	2.7 (0.04)	1.1 (0.00)	0.2 (0.00)	0.1 (0.00)	15.3 (0.04)	75.4 (0.08)	1.1 (0.01)	4.3 (0.01)
Crownvetch								
F1 (n=5)	1.8 (0.42)	1.5 (0.31)	5.1 (0.75)	0.3 (0.64)	4.6 (0.39)	66.9 (4.98)	1.8 (0.27)	18.1 (3.95)
F2 (n=5)	1.2 (0.08)	1.0 (0.11)	3.5 (0.35)	1.7 (0.21)	5.4 (0.10)	66.3 (0.86)	1.8 (0.07)	19.1 (0.31)
F3 (n=1)	0.7	0.7	2.5	1.1	5.7	67.3	1.9	20.3
Tilia sp								
F1 (n=9)	-	-	5.0 (0.34)	36.3 (2.62)	-	3.8 (0.99)	4.6 (0.27)	50.3 (2.45)
F2 (n=9)	-	-	1.8 (0.15)	14.9 (0.73)	-	3.0 (1.03)	2.7 (0.16)	77.7 (1.13)
Weeping willow								
F1 (n=2)	0.72 (1.02)	2.54 (0.28)	18.8 (0.98)	12.9 (0.19)	9.66 (0.88)	12.4 (0.44)	14.4 (0.49)	28.7 (0.39)
F2 (n=2)	0.25 (0.35)	0.52 (0.07)	6.43 (0.87)	4.80 (0.14)	13.4 (4.87)	17.3 (2.47)	16.9 (2.07)	40.4 (5.21)
F3 (n=1)	1.29	1.32	2.90	1.84	16.7	22.5	10.3	43.1
Sainfoin								
F1 (n=2)	8.93 (0.30)	12.1 (2.14)	6.59 (0.83)	7.42 (0.66)	15.1 (1.05)	33.1 (0.36)	3.08 (1.15)	13.7 (2.53)
F2 (n=2)	2.66 (0.34)	2.15 (0.32)	3.13 (0.35)	2.65 (0.28)	10.9 (1.02)	49.0 (1.47)	2.93 (0.06)	26.6 (0.91)
F3 (n=1)	2.32	1.88	2.75	2.35	10.5	50.7	2.78	26.7

*) analytical replicates; "-" not detected; GC = gallocatechin, EGC = epigallocatechin, C = catechin, EC = epicatechin

Table S4. Weight, purity and composition of condensed tannins (CT) from thiolysis degradation of replicate purified (n=2) fractions of sainfoin (2 g crude extract) using Sephadex LH-20 batch chromatography (*Method 4*) (SD from replicate fractionations in parentheses).[a]

Solvent	Yield in grams	Recovered weight % of applied sample	% CT content (g/100 g fraction)	mDP	PC/PD	*cis/trans* flavan-3-ols
1:1 Methanol/water (**F0**)	1.39 (0.01)	69.5	9 (4)	-	7.0/93.0 (0.7)	91.3/8.7 (0.9)
3:7 Acetone/water (**F1**)	0.11 (0.04)	5.4	20 (7)	7.0 (1.4)	14.0/86.0 (4.8)	79.6/20.4 (0.4)
1:1 Acetone/water (**F2**)	0.22 (0.06)	10.7	96 (8)	16.8 (1.0)	12.6/87.4 (0.5)	85.0/15.0 (1.4)
7:3 Acetone/water (**F3**)	0.13 (0.08)	3.8	94 (16)	22.0 (10.0)	12.0/88.0 (1.3)	84.0/14.0 (3.0)
Recovery	1.85	92.5				

[a] % CT content refers to purified fractions;
Abbreviations used: mDP: mean degree of polymerization; PC/PD: molar percentages of procyanidins/prodelphinidins; *cis/trans*: molar percentages of *cis*/*trans* flavan-3-ols.

Table S5. Flavan-3-ol subunits (molar percentages) composition from fractionations purified once using Sephadex LH-20 batch chromatography (*Method 4*). Fractions were analyzed by thiolytic degradation for flavan-3-ol composition.
(SD is from replicate analytical analysis except that of goat willow which is from replicate fractionations)

	Plant species	Fraction	Terminal units (%)				Extension units (%)			
			GC	EGC	C	EC	GC	EGC	C	EC
1	**Birdsfoot trefoil**	F3	-	-	2.41 (0.03)	0.85 (0.05)	0.86 (0.04)	41.6 (0.4)	2.21 (0.05)	52.1 (0.08)
2	**Big trefoil**	F3	2.0 (0.3)	0.59 (0.03)	1.79 (0.01)	0.67 (0.03)	9.13 (0.05)	68.9 (0.5)	2.24 (0.03)	14.7 (0.2)
3	**HT Mediterranean trefoil**	F3	0.5 (0.01)	-	1.98 (0.03)	0.13 (0.01)	1.26 (0.1)	67.0 (0.07)	1.94 (0.02)	24.7 (0.1)
4	**Goat willow**	F2	1.90 (0.3)	0.9 (0.1)	9.71 (0.03)	0.97 (0.04)	42.2 (0.08)	9.90 (0.2)	28.4 (0.1)	6.87 (0.02)
5	**Goat willow**	F3	1.75 (0.09)	-	5.89 (0.013)	0.61 (0.05)	44.1 (0.3)	13.0 (0.3)	26.2 (0.01)	8.45 (0.01)
6	**Black currant**	F2	1.8 (0.1)	1.30(0.07)	1.01 (0.01)	1.01 (0.02)	13.83 (0.04)	13.06 (0.05)	1.46 (0.02)	0.78 (0.06)
7	**Black currant**	F3	5.3 (0.3)	0.7 (0.1)	0.60 (0.01)	0.05 (0.06)	66.9 (0.1)	24.1 (0.1)	1.30 (0.02)	1.06 (0.06)

- not observed
GC = gallocatechin, EGC = epigallocatechin, C = catechin, EC = epicatechin

Table S6. Flavan-3-ol subunits (molar percentages) composition from fractionations purified twice using Sephadex LH-20 batch chromatography (*Method 4*). Fractions were analyzed by thiolytic degradation for flavan-3-ol composition (SD is from replicate analytical analysis except that of HT Mediterranean which is from replicate fractionations).

	Plant species	**Fraction**	**Terminal units (%)**				**Extension units (%)**			
			GC	**EGC**	**C**	**EC**	**GC**	**EGC**	**C**	**EC**
1	**Birdsfoot trefoil**	P2F3	-	-	3.97 (0.02)	1.4 (0.1)	0.87 (0.01)	36.1 (0.4)	2.70 (0.10)	55.0 (0.30)
2	**Big trefoil**	P2F3	2.0 (0.4)	0.7 (0.1)	2.25 (0.03)	0.82 (0.01)	9.52 (0.05)	67.1 (0.4)	2.43 (0.09)	2.06 (0.08)
3	**Big trefoil**	P2F4	1.8 (0.1)	0.7 (0.1)	2.78 (0.03)	1.05 (0.02)	9.98 (0.07)	65.9 (0.3)	2.58 (0.01)	15.2 (0.20)
4	**Sericea lespedeza**	P2F3	4.9 (0.8)	1.52 (0.05)	0.23 (0.00)	0.30 (0.5)	13.5 (0.2)	73.7 (0.6)	0.74 (0.01)	5.13 (0.02)
5	**Sericea lespedeza**	P2F4	6.3 (0.1)	2.0 (0.2)	0.33 (0.03)	0.40 (0.7)	14.2 (0.1)	70.5 (0.2)	0.80 (0.04)	5.40 (0.10)
6	**HT Mediterranean trefoil**	P2F3 (n=2)	1.2 (0.2)	-	2.94 (0.06)	0.20 (0.01)	3.80 (0.09)	63.3 (0.2)	2.38 (0.03)	26.2 (0.10)
7	**HT Mediterranean trefoil**	P2F4 (n=2)	1.2 (0.3)	-	4.22 (0.02)	0.30 (0.01)	4.10 (0.1)	61.0 (0.1)	2.74 (0.04)	26.4 (0.08)
8	**White clover flowers**	P2F3	4.34 (0.05)	1.2 (0.2)	-	-	24.5 (0.2)	69.4 (0.4)	0.26 (0.01)	0.71 (0.01)
9	**White clover flowers**	P2F4	6.4 (0.5)	2.2 (0.2)	-	-	25.4 (0.3)	64.9 (0.3)	0.26 (0.01)	0.80 (0.10)
10	**Black currant leaves**	P2F3	5.45 (0.02)	0.49 (0.08)	0.62 (0.03)	-	12.3 (0.3)	18.5 (0.1)	1.26 (0.01)	0.75 (0.01)
11	**Black currant leaves**	P2F4	8.0 (0.2)	0.9 (0.2)	0.76 (0.03)	-	73.3 (0.3)	15.1 (0.2)	1.31 (0.01)	0.67 (0.07)

- not observed

GC = gallocatechin, EGC = epigallocatechin, C = catechin, EC = epicatechin

Table S7: Summary of conditions suited for isolating different CTs.

Method number & description	Conditions and fraction numbers	Pros	Cons
Method 1: 'Standard Column' Toyopearl HW-50F Chromatography	1. Aqueous acetone (7:3) extract: 2 g 2. Column dimensions: 230 mm x 30 mm 3. Eluents and fractions: - water (300 mL) - methanol/water (1:1; 300 mL) - acetone/water (7:3; 3 x 100 mL): **TF7, TF8 and TF9** - acetone (100 mL): **TF10**	1. mDP: excellent size separation mDP values of 2 to 95 2. PC/PD: some separation of PCs from PDs	1. Yields: 4 mg to 184 mg 2. CT purities: less than <60%
Method 2: 'Standard Column' Chromatography on Toyopearl HW-50F versus Sephadex LH-20	1. Extract (6 g) was pre-purified on Sephadex LH-20 (120 mm x 30 mm) and fraction eluted with the aqueous acetone fraction (7:3; _380 mg) was loaded on either column: 2. Toyopearl or Sephadex column dimensions: 185 mm x 30 mm 3. Eluents and fractions: - water: 3 x 100 mL: **T1 to T3** or **S1 to S3** - methanol/water (1:1; 3 x 100 mL): **T4 to T6** or **S4 to S6** - acetone/water 7:3; 100, 50, 50, 100 mL): **T7, T8a, T8b, T9** or **S7, S8a, S8b, S9** - acetone (100 mL): **T10** or **S10**	1. CT purities of aqueous acetone fraction: >80% 2. Toyopearl HW-50F better than Sephadex LH-20 at separating CTs according to mDP values and PC/PD ratios	1. Pre-purification step essential to achieve high CT purities. 2. Yields: 3 mg to 119 mg 3. Time needed: ca 5 hours
Method 3: 'Wide column' Sephadex LH-20 Chromatography	1. Aqueous acetone (7:3) extract: 10 g 2. Sephadex LH-20 (50 g) 3. Column dimensions: 70 mm x 65 mm 4. Eluents and fractions: - water (2 L) - acetone/water (3:7): **F1** - acetone/water (1:1): **F2** - acetone/water (8:2): **F3**	1. Yields of Fraction 1: 146 to 800 mg; yields of Fraction 2: 174 mg to 1200 mg 2. CT purities of Fraction 2: 64% to 100% 3. mDP ranges: 3 to 12 (Fraction 1); 8 to 18 (Fraction 2); 14 to 30 (Fraction 3)	1. PC/PD ratios: little separation of PCs and PDs 2. Fraction 3 gives CTs with higher mDP values but low yields

		4. Time needed per run: 4 to 5 hours 5. Sephadex LH-20 (50 g) column can be re-used up to 10 times	
Method 4: 'Batch Chromatography' with Sephadex LH-20.	1. Aqueous acetone extract: 4.5 g to 19 g 2. Sephadex LH-20 (10 g per g of extract) 3. Buchner filter funnel 600 or 1500 mL 4. Eluents and fractions: - methanol/water (1:1; 3 x 5 mL/g Sephadex LH-20): **F0** - acetone/water (3:7; 3x 5mL/g Sephadex LH-20): **F1** - acetone/water (1:1; 3x 5mL/g Sephadex LH-20): **F2** - acetone/water (7:3; 3x 5mL/g Sephadex LH-20): **F3**	1. Yields of **F3** and **F4**: 100 mg to 1.23 g 2. CT purities: 73 to 97% 3. mDP ranges: 8 to 38 4. Time needed per run: 2.5 hours	1. Sephadex LH-20 is used once

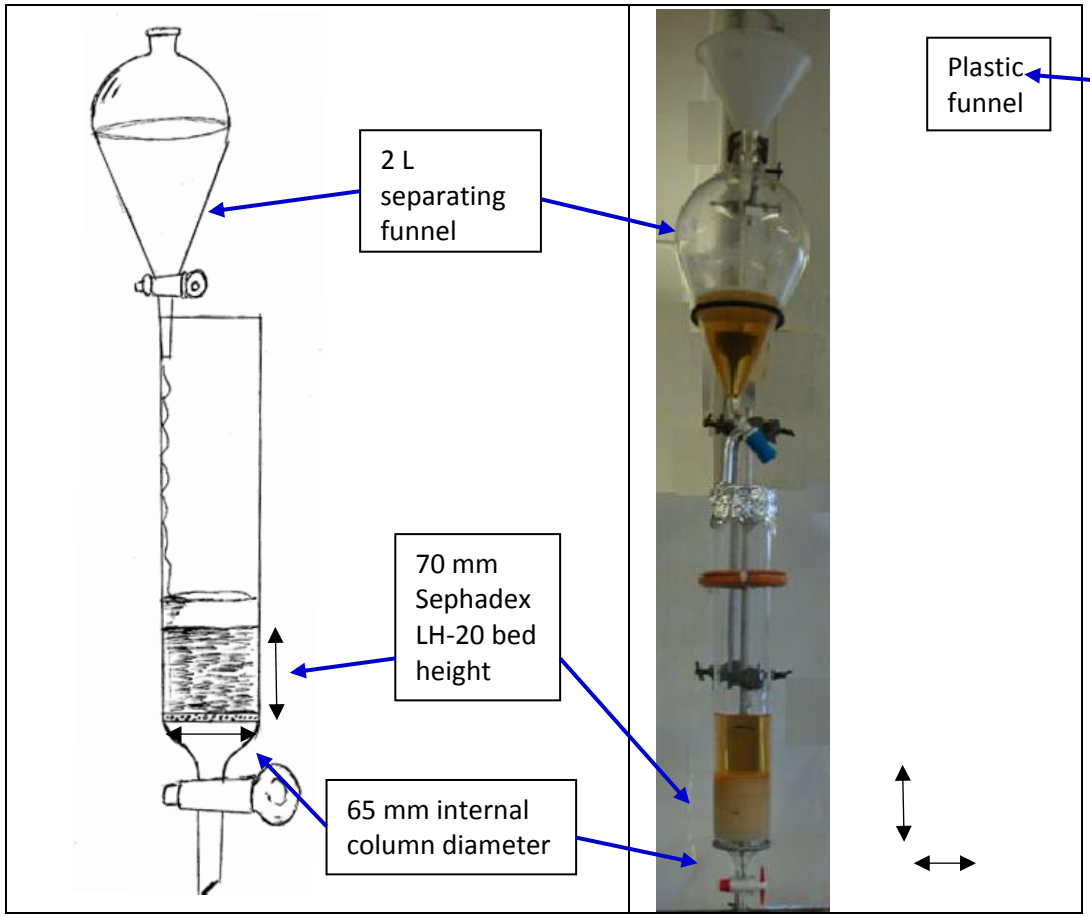

Figure S1. Set up for wide Sephadex LH-20 column fractionation (*Method 3*).