P. trichocarpa Crude Protein Isolation

Supply and Reagents:

Extraction buffer: 20ml (based on 3g-5g tissue in 20ml buffer)

Final conc	take
50mM Tris-HCl pH 7.6 (or pH8)	20ml
Sodium Ascorbate 20mM	0.07924g
Sucrose 0.4M	2.736g
NaCl 100mM	
1M DTT 5mM	100ul

Add following before extraction:

Polyvinylpolypyrrolidone(PVPP) 10% w/w	500mg	
1M PMSF (in DMSO) 1 mM		20 ul
1mg/mL pepstatin A (in MeOH) 1ug/ml	20 ul	
1mg/mL leupeptin (in H2O) 1ug/ml	20 ul	

Notes: If the protein is used for MS proteomics analysis, do not use PMSF, pepstatin A ,and leupeptin.

Protocols:

1. Grind the sample with a coffee grinder
2. Transfer the powderized tissues into two extraction tube containing 15ml E buffer each and (freshly add PVPP AT, PMSF, pepstatine, leupeptine)
   Notes: when the protein is used for enzyme assay, add 20% glycerol in the extraction buffer
3. Use Bio-homogenizer 30s, then incubate in ice for 1 min, 6-8 times
4. Centrifuge 3000g for 15min, centrifuge supernatant 3000g for 15min or:6000Xg for 30min, filter with miracloth
5. Supernatant used for protein concentration
6. Sample (15ul) mixed with 2X loading buffer(15ul), heat 100 degree for 10min before loading, load 15ul.
7. The crude can be used for enzyme assay
   The crude protein can be stored to -80 immediately