

Extraction of Phenolics from Plants

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The method used to extract phenolics must be planned with care since all results of the analyses are affected by the extraction step. Solvent and method of homogenization affect both recovery of extraction and selectivity of extraction. Some solvents are incompatible with some methods of analyses. In particular, acetone inhibits all protein precipitation assays except the radial diffusion assay. Crude plant extracts are not chemically stable, and can change rapidly during aging. Phenolics can be extracted from fresh, frozen, dried or lyophilized plant samples. Sample treatment does affect phenolic extractability, so all samples should be treated similarly [Hagerman, *J. Chem. Ecol.* 14, 453-461 (1988); Cork and Krockenberger, *J. Chem. Ecol.* 17, 123-134 (1991); Orians, C.M. *J. Chem. Ecol.* 21, 1235 (1995); Lindroth, R.L.; Koss, P.A. *J. Chem. Ecol.* 22, 765 (1996).

Lyophilizing tissue

Dry tissue is easy to handle, but phenolics are frequently not stable to sun drying or drying in an oven. To conveniently lyophilize, or freeze dry, plant tissue, cut the fresh tissue into small pieces with scissors, and place it in a mortar with liquid nitrogen. Grind with the pestle to yield a fine powder, keeping it frozen with liquid nitrogen throughout. Put the sample in a bag made of Miracloth, and lyophilize immediately. (When samples are lyophilized, or freeze dried, water is removed by sublimation. A variety of types of lyophilizers can be obtained from makers of refrigeration or vacuum equipment).

Extraction with a sonicator

A sonicator uses high energy sound waves to force solvent to penetrate "unwetable" material. In a sonicating bath, multiple samples can easily be handled at once. Use about 2 g dry weight or 8 g wet weight of the plant tissue. If using fresh or frozen tissue, grind it first with liquid nitrogen with a mortar and pestle. Transfer the sample to a 50 mL screw cap plastic centrifuge tube and record the sample weight. Add 20.0 mL 70% acetone (acetone:water, 70:30) and sonicate 30 min at 4 C. Centrifuge 10 min at 2500 x g. Remove supernatant and record exact volume. Save supernatant at 4 C. Repeat the extraction three more times. We then usually combine the first two extractions (about 75 % of the total phenolics) and the second two extractions (about 20 % of the total phenolics) for analyses.

Extraction with a homogenizer

Torti et al. [*J. Chem. Ecol.* 21, 117-125 (1995)] showed that for some samples phenolics can be extracted efficiently and reproducibly using a Polytron-type homogenizer. Care must be taken not to cross contaminate samples with the homogenizer blade, which must be cleaned thoroughly to remove traces of plant debris. "Stringy" samples are difficult to homogenize, and the volume of liquid must be fairly large to accommodate the blade.