# Chapter 18
# Total Phenolics


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## 1 Introduction

Phenolics are a heterogeneous group of natural substances characterized by an aromatic ring with one or more hydroxyl groups. The number of these compounds identified may now exceed 100,000 (Waterman and Mole 1994; Tissier et al. 2015). Phenolics may occur as monomers with one hydroxyl group (e.g., ferulic acid). Compounds with several phenolic hydroxyl substituents are referred to as polyphenolics (Harborne 2004). Among these, tannins (subdivided into phlorotannins, hydrolyzable and condensed tannins) are of particular interest because of various demonstrated or posited ecological effects (Zucker 1983; Chap. 19). In particular, tannins play a major role in the defense against herbivores and pathogens (Waterman and Mole 1994; Lill and Marquis 2001) or, more generally, in communications between plants and other species (Harborne 2004; Preiss et al. 2015). Other phenolics such as anthocyanins may prevent leaf damage resulting from exposure to excessive light (Gould and Lee 2002).

Larger phenolics are often concentrated in specific tissues or cell structures (e.g., leaves, bark), and overall concentrations of phenolics in green leaves vary widely within a range of 1–25% of dry mass (Hättenschwiler and Vitousek 2000). Since the bulk of phenolics remains present during leaf senescence and after death, these

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**Table 18.1** Phenolics concentrations in terms of tannic acid or ferulic acid equivalents for selected plant tissues, including senescent leaves (s) and live (l) and yellow-green to brown-dead grass leaves (y)

| Species | Common name | Phenolics (% leaf dry mass) | References |
|---|---|---|---|
| *Spartina alterniflora* (y) | Smooth cordgrass | 0.4–1.5 | 1 |
| | | 0.2–1.2 | 2 |
| *Qualea* sp. (s) | | 2.7 | 3 |
| *Alnus glutinosa* (s) | Alder | 2.7 | 4 |
| | | 2.7 | 5 |
| | | 6.6 | 6 |
| | | 6.8–7.6 | |
| *Sapium sebiferum* (l, s) | Chinese tallow tree | 3.0 | 7 |
| *Eucalyptus globulus* (s) | Eucalyptus | 6.4 | 4 |
| | | 9.8 | 5 |
| *Fagus sylvatica* (s) | Beech | 8.0 | 7 |
| *Carya glabra* (s) | Hickory | 9.1 | 9 |
| *Platonia* (s) | | 12.5 | 3 |
| *Quercus alba* (s) | Oak | 16.2 | 9 |
| *Acer saccharum* (s) | Sugar maple | 15 | 8 |

1 = Graça et al. (2000); 2 = Bärlocher and Newell (1994); 3 Coq et al. 2010; 4 = Pereira et al. (1998); 4 = Bärlocher et al. (1995); 6 = Gessner (1991); 7 = Cameron and LaPoint (1978); 8 = Graça and Bärlocher (1998); 9 = Suberkropp et al. (1976)

compounds may also affect microbial decomposers (Harrison 1971) and litter-consuming detritivores and therefore delay decomposition of plant litter (Zucker 1983; Salusso 2000). The amount of phenolics in plant tissues varies with leaf species, age, and decomposition stage. Values for selected plants are summarized in Table 18.1.

A first step in many studies assessing the ecological effects of phenolics is an estimate of the total concentration of phenolic hydroxyl groups. To this end, the material is typically dried and extracted with water or, more commonly, with organic solvents such as acetone or methanol. Pre-drying may affect the efficiency of the extraction process (Chap. 5). In green or senescing material, polyphenol oxidases (PPO) may degrade phenolics. This can be prevented by PPO inhibitors like $K_2S_2O_4$ or by using liquid nitrogen or by boiling the sample for a few seconds to inactivate the enzymes. Solid-phase microextraction, pressurized liquid or fluid extraction, and microwave-assisted extraction are alternatives to conventional solvent extraction (Ainsworth and Gillespie 2007; Ajila et al. 2011).

The most commonly used approach to measure phenolics in the extract was originally designed to quantify the phenolic amino acid tyrosine (Folin and Denis 1912). Folin and Ciocalteu (1927) made the assay more sensitive and less prone to formation of precipitates. Preparation of the Folin-Denis or Folin-Ciocalteu reagent is relatively time-consuming, but these reagents are now commercially available (Waterman and Mole 1994). Here we present the procedure introduced by Folin and Ciocalteu (1927). The Folin-Ciocalteu assay is relatively non-specific, and other

reductants in the extract may be inhibitory, additive, or enhance the reaction; for example, additive effects may occur in the presence of aromatic amines, high sugar levels, or ascorbic acid (Ainsworth and Gillespie 2007). More sophisticated approaches to measure phenolics include HPLC, GC, LC-MS, AFT-IR, and NMR (Ajila et al. 2011).

## 2 Equipment, Chemicals, and Solutions

### *2.1 Equipment and Material*

- Eppendorf pipettes
- Vortex
- Refrigerator
- Dried leaves
- Mill or mortar and pestle
- Analytical balance (±0.1 mg precision)
- Eppendorf tubes
- Centrifuge
- Spectrophotometer

### *2.2 Chemicals*

- Tannic acid standard (other phenolics such as gallic acid could also be used)
- Acetone
- Deionized water
- 2% $Na_2CO_3$
- 0.1 M NaOH
- Folin-Ciocalteu reagent (e.g., Sigma F-9252; diluted 1:2 with deionized water).

## 3 Experimental Procedures

### *3.1 Calibration*

1. Prepare a stock solution of 25 mg tannic acid in 100 ml of acetone (30% water, 70% acetone).
2. Transfer 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock solution into six Eppendorf tubes and add 1.0, 0.8, 0.6, 0.4, 0.2, and 0 ml of distilled water, respectively. Mix with vortex.

3. Add 5 ml of 2% $Na_2C0_3$ in 0.1 M NaOH and mix.
4. After 5 min, add 0.5 ml of Folin-Ciocalteu reagent and mix.
5. After 120 min, read absorbance at 760 nm.
6. Plot tannic acid concentration vs. absorbance. The relationship should be linear.

### *3.2 Measurement*

1. Grind dried leaves to powder passing through a 0.5 mm mesh size screen.
2. Weigh out approximately 100 mg portions of the ground leaves and transfer to Eppendorf tubes.
3. Extract phenolics in 5 ml of 70% acetone for 1 h at 4 °C.
4. Centrifuge (10,000–20,000 g, 10–20 min).
5. Take 0.5 ml of the supernatant (or another value between 0.1 and 0.8), and make up to 1 ml with distilled water as above.
6. Add $Na_2CO_3$ and Folin-Ciocalteu reagent as above.
7. After 120 min, read absorbance at 760 nm.
8. Based on the standard curve, determine tannic acid equivalents per mg of leaf powder. Remember that in Step 5, only a fraction (0.5 ml) of the sample was used.

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