**Total Flavonoid and Total Phenolic Content Protocol**

*This protocol uses the aluminum complexation reaction for total flavonoids (Pekal et al, 2014) and the Folin-Ciocalteu reaction for total phenolics (Pekal et al, 2014; Singleton et al, 1999). Adapted in part from Kamal (2011).*

\*\*\*This protocol is designed to be with snap-frozen leaf tissue that has been ground into a powder with liquid nitrogen and kept at -80°C. It can be easily modified to work with dried powdered leaf tissue.

(1) Sample tubes need to be weighed to determine sample mass. Should be around 0.05g fresh mass.

(2) Add 1.25ml methanol, allow to extract in the refrigerator for 12-48 hours.

(3) Centrifuge extracts to remove detritus at 3000 rpm for 10 minutes.

*This extract can then be used for both protocols.*

Phenolic protocol:

(1) Add 0.1ml raw leaf extract to 0.9ml water (dilution step).

(2) Add 0.1ml Folin-Ciocalteu reagent and 0.9ml water.

(3) Let stand for 5 minutes.

(4) Add 1ml of 7% (w/v) Na2CO3 and 0.4ml water.

(5) Allow 30 minutes for blue color to form.

(6) Measure absorbance at 765nm.

Flavonoid protocol:  
  
(1)Add 0.2ml raw leaf extract to 1.8ml water (dilution step).  
  
(2) Add 0.2ml of 10% (w/v) AlCl3 and 1ml of water.

(3) Vortex mixture, let stand at room temperature for 10 minutes.  
  
(4) Measure absorbance at 425nm