Bulk Purification of Leaf Tannins
(Appel’s protocol revised 1-09-01 by DD)

I. Preparation for Purifying:
   a. If you have not done so, rotovap the 125ml of leaf extract to remove the acetone or methanol. It’s best to do the extraction in 80% EtOH.
   b. In the refrigerator you will find 4 - 250ml Nalgene bottles containing sephadex LH20 stored in 80% ethanol.
   c. Measure out the rotovaped leaf extract into 4 equal parts (minus any leaf material)

II. Removing Nonphenolics:
   a. Add the leaf extract to the 4 bottles of sephadex.
   b. Shake by hand to break up sephadex pellet.
   c. Place the 4 bottles containing leaf material and sephadex on the shaker and set timer for 5 minutes.
   d. At the end of 5 minutes place the 4 bottles in the centrifuge for 10 minutes.
   e. After 10 minutes remove bottles and test supernate for positive results with the Folin-Denis spot test.(positive is indicated by a dark green color). Do this by adding 20 ul of supernatant to a test tube. To the supernatant add .5 mls of di H2O + 750 uls of Folis-Coicalteu solution + 750 uls (0.5 M )NaCO3 solution. If phenolics are present solution will turn green. Compare the color to a control that contains all the reagents but no tannins. Control will be yellowish.
   f. Discard the ethanol from above the sephadex pellet.
   g. Add 150ml of 95% ethanol to each of the 4 Nalgene bottles and repeat steps b-g until folin-denis test gives a negative result. (stays yellow)

III. Removing Tannins from Sephadex:
   a. After negative result is achieved add 150ml of 70/30 acetone to each of the 4 Nalgene bottles.
   b. Shake the bottles to break up the sephadex pellet.
   c. Place the 4 bottles on the shaker and set timer for 5 minutes.
   d. At the end of 5 minutes, place the 4 bottles in the centrifuge for 10 minutes.
   e. After 10 minutes remove the bottles and test the supernatant for positive results for tannins with the folin-denis spot test.
   f. Remove and save the 70/30 acetone wash. Pour acetone/tannin solution into a clean, labeled storage container and place it in the refrigerator.
   g. Add 150ml 70/30 acetone solution to each of the 4 bottles and repeat steps b-g until folin-denis test gives a negative result.

IV. Cleaning the Sephadex:
   a. Once a negative result has been achieved, add 100ml of 80% ethanol to each of the 4 nalgene bottles, shake to break up the pellet, shake on the machine for 5 minutes and spin the bottles for 10 minutes. ***This step is to remove any
acetone residue from the sephedex.
b. Add **120ml 80% ethanol** to each of the 4 bottles and place them in the refrigerator for storage.