

**Protocol for determining the anti-bacterial properties of naturally occurring substances:**

1. With a mortar and pestle, grind up the substance in a phosphate buffer (pH of ~7.0).
2. Filter the solution (gross filtration) using a double thickness of cheesecloth.
3. Using a syringe, force the filtrate from #2 through a 0.2 micron filter into a sterile tube (fine filtration).
4. Keep this second filtrate cold and aseptic (metal cover over test tube).
5. Perform a serial dilution of the filtrate, resulting in concentrations of full strength (FS), 1/10 FS, 1/100 FS, 1/1000 FS.
6. To 3 ml. of liquid LB (or NA) medium in each of 4 sterile test tubes, add 0.1 ml. of an overnight *E. coli* culture and 0.1 ml. of each of the 4 concentrations of filtrate (i.e., one of each of the 4 different filtrate concentrations for the 4 different test tubes). To a fifth test tube, add only 0.1 ml of overnight culture (the control).
7. After ~6 hrs. take an optical density (OD) measurement of each tube--read the absorbance of the solution at a wavelength of 600 in the spectrophotometer using the LB (or NA) medium as a blank.
8. Take another OD measurement after 24 hours.

Alternatively, you can plate 100 microliters of the bacterial solutions onto LB (or NA) plates using appropriate dilutions (range of 1/1000 to 1/100000 at 6 hrs., and perhaps 1/10,000 to 1/1,000,000 at 24 hrs).