

Catechu extract amount standardization in media preparation

Protocol:1) Preparation of stock solution of catechu extract to be added in media by membrane filter sterilisation.

- 1) Boil around 150 ml of distilled water and take 100 ml of this in a beaker using a standard measuring flask.
- 2) Add 10g of catechu to this boiling water.
- 3) Mix it well and boil the water again for 5 minutes.
- 4) Cool the catechu solution in an ice bath.
- 5) After it is cooled, add 1g of slaked lime and mix it well till all the slaked lime reacts with the catechu.
- 6) Centrifuge this solution at 5000 rpm for 15 minutes and collect the supernatant and again centrifuge it at 5000 rpm for 15 minutes to get rid of suspended particles.
- 7) The supernatant is your stock solution of catechu extract.
- 8) Add 10 ml of this solution to 100ml of media.
- 9) Sterilization of catechu stock:
Using a membrane filter having pore size of 0.22 micron filter the catechu extract through a sterile membrane filtration assembly in a LAF.

Protocol 2) Preparation of stock solution of catechu extract to be added in media after autoclaving and to check stability of absorbance of the extract in different batches.

1)Preparing fine powdered catechu-

- (1) Crush 100 grams of Catechu (bought from Mahalakshmi store, Matunga station) in a mortar and pestle.
- (2) Sieve the ground Catechu through a strainer to obtain fine powdered Catechu.

2) Extraction-

- (1) Boil 150 ml of Distilled water in a beaker placing on a wire gauze.
- (2) In another beaker measure 10 g of fine powdered catechu.
- (3) Measure 100 ml of the boiling water in a 100 ml standard flask.
- (4) Transfer this boiling water to the beaker containing 10 g weighed catechu.
- (5) Stir till the complete powder is to form a homogeneous colloidal mixture.
- (6) Keep this mixture for 5 mins till just boiling with continuous stirring with glass rod at equal intervals of 1 min.
- (7) Turn off the burner and let the mixture cool at room temperature (approx 15-20 mins).
- (8) Prepare an ice bath with 2 ice packs and around 1/4th filled water in a water bath container.
- (9) Place the mixture now at room temperature in the ice bath {to allow maximum dissolution of slake lime - $\text{Ca(OH)}_2 + \text{H}_2\text{O}$ is an exothermic process}

3) Preparing Catechu-Slake lime mixture

- (1) Weigh 1 g of slake lime (Raja Chuna from Matunga station) and add it to the beaker containing extracted Catechu in water mixture.
- (2) Let the mixture react for around 15-30 mins with slake lime till the visible white particles are clear in the liquid.
- (3) Stir in between to check the dissolution of the slake lime particles.

4) Obtaining clear C-SL solution

- (1) The prepared C-SL mixture in ice bath is stirred and poured in clean and dried 15 ml plastic centrifuge tubes.
- (2) Adjust the weight of the tubes and centrifuge them for 15 mins at 5000 rpm at room temperature.
- (3) Decant the supernatant in different 15 ml plastic centrifuge tubes and centrifuge again for 15 mins at 5000 rpm. {Second centrifugation is for confirming all the particulate matter is separated from the mixture and clear solution is obtained.}
- (4) Decant the obtained clear supernatant from second centrifugation in a glass jar bottle.

5) Standardizing C-SL solution

- (1) The obtained clear C-SL solution is autoclaved for sterilization.
- (2) Take 3 ml of this solution aseptically and check for the Optical density.
- (3) Dilutions of the catechu stock solution in triplicates are prepared of 0.1%, 0.5% and 1% conc. with total volume 5 ml and blank used Distilled water.
- (4) The OD of the solution measured at 254 nm wavelength should be checked for each preparation and then used in the medium.