

## 13.

### TLC Bioautography Methods - Antifungal Activity

TLC bioautography methods are important in the search for bioactive compounds, based on localization of active compounds on a TLC chromatogram. This method can be used to screen especially antifungal, antibacterial, antioxidant activity etc qualitatively. Simplicity, speed and also low cost are the advantages of this method.

#### Screening for Antifungal Activity

##### *Preparation of Potato Dextrose Agar medium (PDA)*

Cook sliced potatoes (200 g) in water (1 L). Filter and add dextrose (20 g) and agar (20 g). Add distilled water to make up final volume to 1 L and autoclave at 121°C for 20 min. After 20 min, allow the PDA medium to cool to 35-40 °C and pour into sterilized Petri-dishes under aseptic condition. Inoculate the solidified PDA with *Cladosporium cladosporioides*.

##### *Preparation of Czapek-Dox Medium*

Dissolve KNO<sub>3</sub> (1 g), K<sub>2</sub>HPO<sub>4</sub> (1 g), MgSO<sub>4</sub> (0.25 g), KCl (0.25 g), FeSO<sub>4</sub> (0.005 g) and sucrose (15 g) distilled water (500 ml) and autoclave for 20 minutes at 121°C.

##### *Preparation of the Spore Suspension*

Pour sterilized Czapek-Dox medium (10 ml) into two cultures of previously grown *C. cladosporioides* on Petri-dish under aseptic condition and shake well. Filter the spore suspension into a spraying bottle through double muslin cloth. Mycelia retain in the muslin cloth while the spore carrying nutrient medium filter in to the spraying bottle.

##### *Test*

After developing and drying, TLC plates are spray with *Cladosporium cladosporioides* spore suspension. Place inoculated plates in a humid chamber for 4 days at 25°C in darkness to monitor the antifungal activity of compounds on the TLC. Clear zones of fungal growth inhibition indicate the presence of antifungal constituents. (Figure 1)

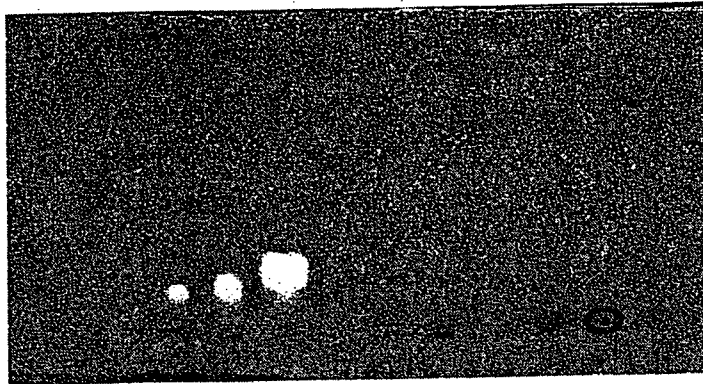


Figure 1: TLC bioautography (*Cladosporium cladosporioides*) of a column fractions collected from a plant extract

#### References

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