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Technical Report: A rapid method for detecting fungi-toxic substances

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A simple and rapid technique is reported for the preliminary screening of fungi-toxic extracts/samples by direct spotting onto silica gel plates and subsequent over-spraying with a fungal spore suspension. After incubation fungi-toxicity is indicated by a growth inhibition zone, the area of which is related to the concentration of the sample.

Key words: *Adenocalymma alliaceum*, *Alternaria brassicae*, antifungal activity, *Aspergillus niger*, spore germination.

Several methods for detecting anti-fungal activity have been reported (Homans & Fuchs 1970; Pero & Owens 1971) and reviewed (Janssen *et al.* 1987; Paxton 1991). A method for studying fungal growth inhibition by plant proteins was developed by Ludwig & Boller (1990). During screening of plant extracts for anti-fungal activity, the main difficulty was their non-miscibility with water. The agar diffusion assay is time-consuming, as is the bioautographic method (Homans & Fuchs 1970), which involves the development of each sample on TLC plates in a suitable solvent system. We report here a rapid method based on that of Homans & Fuchs (1970) allowing screening of large numbers of samples for anti-fungal activity.

Materials and Methods

Plant Extracts

Extracts of *Adenocalymma alliaceum* Miers leaves were used in this study. Fresh leaves were extracted with petroleum ether (b.p. 60–80°C) by homogenizing in a Waring blender for 1 min. The homogenate was incubated at room temperature (25 to 30°C) for 2 days and then filtered through Whatman No. 1 filter paper. The solvent was evaporated under reduced pressure and the residue then re-dissolved in petroleum ether before testing for anti-fungal activity.

Technique

The extracts were applied to silica gel thin-layer plates, dried at room temperature, and over-sprayed with 2 ml of spore suspension (10^7 spores/ml) of the test fungi *Alternaria brassicae* and *Aspergillus niger* in Czapek-Dox medium ($NaNO_3$, 2 g; K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 0.5 g; KCl 0.5 g; $FeSO_4.7H_2O$, 0.01 g; sucrose 30 g; distilled water 1 l). The plates were incubated in a humid chamber at 25 ± 2°C for 3 to 4 days until growth of the fungus became visible. A control plate spotted with the corresponding organic solvent was run in parallel.

Results and Discussion

The petroleum ether extract of *Adenocalymma alliaceum* leaves exhibited a clear zone of growth inhibition when tested against *A. brassicae* and *Asp. niger*. (Figure 1). The area of growth inhibition is directly related to the quantity

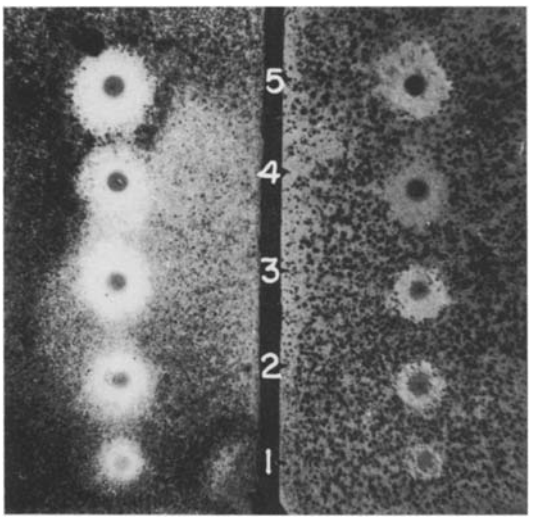

Figure 1. Inhibition of *Alternaria brassicae* (left) and *Aspergillus niger* (right) spore germination by a petroleum ether extract of *Adenocalymma alliaceum* leaves. Aliquots containing 50, 100, 150, 200 and 250 μg crude extract were applied to silica gel plates at points 1, 2, 3, 4 and 5 respectively. The plates were then treated as described.

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of the testing material; increased amounts of the extracts resulted in an increased area of growth inhibition (Figure 1). Inhibition of fungal growth was not observed on the control plates which were spotted with petroleum ether, chloroform or methanol. The method exhibited good sensitivity for crude plant extracts in these different solvents, and is applicable to the testing of liquid column chromatography eluates for fungi-toxic activity. This method could possibly be applied to other assays presently based on agar diffusion, such as the identification of mutants resistant to certain substance(s) (Fromtling & Bulmer 1978), and for testing fungicidal/fungistatic action samples (Garber & Houston 1959).

Conclusion

The technique reported herein is a simple and rapid alternative for the preliminary screening and later purification of anti-fungal compounds from plant extracts.

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