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Working

Detection of anti-phytopathogenic fungal activity

Version 2
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Cecilia Monica Rodriguez García[1], Leticia Peraza Echeverría[1]
[1]Centro de Investigación Científica de Yucatán

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Leticia Peraza Echeverría
Centro de Investigación Científica de Yucatán

ABSTRACT

Here we describe the detection of in vitro anti-phytopathogenic fungal activity using the broth dilution method to find the MIC (minimum inhibitory concentration) of an aqueous extract of leaves. Four concentrations were assayed: 1, 2.5, 5, and 10% (v/v) in PDB medium. Each concentration was inoculated with a spore suspension of the selected fungus. PDB without spores was used as negative control and with the addition of a commercial fungicide like positive control. After the inoculation of the fungus, the tubes were incubated for three days in the dark at 26-28 °C. After the incubation time, the tubes were checked for growth of the fungus, if no growth is observed, 25 µL of the suspension are transferred to PDA medium in Petri dishes of 3 cm diameter. Evaluations were made on day 7. The MIC was determined as the lowest concentration of aqueous extract preventing the growth of macroscopically visible mycelium.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rodríguez-García CM, Ruiz-Ruiz JC, Peraza-Echeverría L, Peraza-Sánchez SR, Torres-Tapia LW, Pérez-Brito D, Tapia-Tussell R, Herrera-Chalé FG, Segura-Campos MR, Quijano-Ramayo A, Ramón-Sierra JM, Ortiz-Vázquez E (2019) Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. PLoS ONE 14(3): e0213493. doi: 10.1371/journal.pone.0213493

PROTOCOL STATUS

Working

GUIDELINES

- 35 x 10 mm polystyrene Petri dish from Corning
- Commercial fungicide (Miconazole nitrate, 20 mg/L)
- Culture room for the fungal growth (26 + 2 °C at 12/12 h photoperiod)
- Microscope Axioplan from Zeiss
- Everything must be done in a laminar flow hood

MATERIALS

NAME	CATALOG #	VENDOR
Potato Dextrose Agar, Difco	DF0013-15-8	Fisher Scientific
Potato Dextrose Broth, Difco	DF0549-17-9	Fisher Scientific

SAFETY WARNINGS

BEFORE STARTING

- Petri dishes with PDA medium

- PDB medium
- Everything should be sterilized

Dilution of the aqueous extract of leaves

1

- Performs three dilutions from the 10 % aqueous extract (AE)

Dilution number	AE (µL)	CS (µL)	H_2O (µL)	final volume (µL)	concentration (%)
1	500	100	400	1000	5
2	250	100	650	1000	2.5
3	125	100	775	1000	1.25
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CS- conidial suspension (200 conidium/µL)

Do not add de CS, only make the mix of AE and water, CS is added tube by tube

Dilution of the aqueous extract of leaves

2

1. Add to a sterile 1.5 mL eppendorf tube mix 1 and then 12.5 µL of CS (in duplicate)
2. Repeat num 1 for the next concentratión and so on..
3. For the 10% AE just put in each tube 125 µL 10% AE plus 12.5 µL of CS, (in duplicate)
4. The positive control is prepared by adding 1 µL of a commercial fungicide to 125 µL of water and 12.5 µL of CS (in duplicate)
5. Two negative controls: one is only 125 µL of water and the other is 125 µL of PDB and both with 12.5 µL of CS (in duplicate)

Bioassay conditions

3 Once the conidial suspension has been added, homogenize very well, seal the tubes and leave them in the dark at a temperature of 26-28 ° C for 3 days.

4 After three days, 25 µL of the suspension is transferred in the center of the PDA medium (polystyrene dish, Corning), in duplicate.

The suspension of each eppendorf tube must be perfectly homogenized

5

- Petri dishes are incubated at 26 ± 2 °C at 12/12 photoperiod for 7 days (two replicates /concentration)
- Evaluation are made on day 7

If the aqueous extract of leaves is fungicidal there should be no growth on the PDA medium, if the extract is not fungicidal then there must be growth of the fungus on the PDA medium