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Working

Preparation of aqueous extracts of plants [↗](#)

PLOS One

Cecilia Mónica Rodríguez García¹, [Leticia Peraza](#)¹

¹Centro de Investigación Científica de Yucatán

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Leticia Peraza Echeverría
Centro de Investigación Científica de Yucatán



ABSTRACT

Aqueous extracts of plants are a simple, economical and eco-friendly alternative to use as a source of antifungal activity. Here is a protocol for extracting any type of plant material (leaves) with water using a simple blender to grind the dried leaves with water and then a series of centrifuges to eliminate the solid waste, finally a series of filtrations to have an aqueous extract sterile. This aqueous extract can be stored at 4 ° C and depending on the plant the activity can be maintained for a fixed period of time. After this process the aqueous extract is ready to be used in bioassays of antifungal activity.

EXTERNAL LINK

[10.4172/2157-7471.1000332](https://doi.org/10.4172/2157-7471.1000332)

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Ruiz-Ruiz JC, Peraza-Echeverría L, Soto-Hernández RM, San Miguel-Chávez R, Pérez-Brito D, et al. (2016) *Diospyros cuneata* Inhibition of *Fusarium oxysporum*. Aqueous Extract and its Encapsulation by Ionic Gelation. J Plant Pathol Microbiol 7: 332.

PROTOCOL STATUS

Working

GUIDELINES

- 1.- Falcon tubes are reused (clean)
- 2.- Eppendorf tubes are reused (clean)
- 3.- Filter holder (swinnex)
- 4.- Durapore, Membrane filters from Millipore (25 mm)
 - 0.65 µm DV
 - 0.45 µm HV
 - 0.22 µm GV
- 5.- Stove with 50 ° C temperature capacity

MATERIALS

NAME

CATALOG #

VENDOR

Water refers to sterilized deionized water

SAFETY WARNINGS

Carry out all the steps without stopping

BEFORE STARTING

Sterilize: water, filter holder (swinnex) prepared with the 0.22 µm GV

Aqueous extract preparation from leaves

- 1 -Collection and preparation of leaves for drying
 1. Collect fresh leaves from the field

2. In the lab wash them with tap water
3. Remove excess water with a kitchen centrifuge

2 -Drying of leaves

1. Distribute the leaves in cardboard boxes
2. Put them at 50 °C in a stove for 2 -3 days
3. Store them in plastic bags until use

3 1. Blend in a laboratory blender at the maximum speed

 **10 g dry leaves**

 **00:01:30 every 30 sec stir with a spatula**

4 2. Add

 **100 ml sterile distilled water**

 **00:01:30 mix everything**

5 3. Pour the mixture into 50 mL falcon tubes and centrifuge

 **00:30:00 at 3220 g**

 **20 °C**

6 4. Take the supernatant and pour it into a container, take 1800 µL in 2 mL eppendorf tubes, use the necessary tubes 5. centrifuge

 **00:30:00 at 20817 g, room temperature**

7 6. Take the supernatant of all the tubes and pour it into a container 7. Filter the aqueous extract with the membrane of 0.65 µm contained in a swinnex 8. Take the previous filtering and filter it with the membrane of 0.45 µm contained in a swinnex

8 9. In a laminar flow hood, sterilize the aqueous extract by filtration using a membrane of 0.22 µm contained in a swinnex 10. Store the aqueous extract in a sterile 50 mL falcon tube in the dark at 4 ° C until its use.



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