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Working

Assessment of antimicrobial activity

Version 2

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ABSTRACT

The indiscriminate use of antibiotics is one of the main factors that cause the emergence of resistant microorganisms, and it has become a global health problem. That is why the need to search for new compounds with antimicrobial potential arises. One of the most widely used techniques for evaluating the antimicrobial potential of different compounds is the Disk diffusion method, which evaluates the qualitative and semi-quantitative antimicrobial potential of compounds from different natures, having as principle the diffusion of the compounds on agar matrix with elements (culture medium) that allow the growth of microorganism. On the other hand, a method that complements the antimicrobial analysis is broth microdilution, which is a quantitative method. This method allows us to determine the minimum concentration required to inhibit the growth of any microorganism, which in turn helps to determine appropriate doses to evaluate in further methodologies.

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

SAFETY WARNINGS

1 Bacterial and Fungal material

Human pathogenic strains were employed to test the antimicrobial activity of plant extracts; *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Vibrio cholera* ATCC 14035, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 25923. The fungus employed in this work was *Candida albicans* ATCC 1023. All strains were grown in Luria Broth at 37 °C and stored in glycerol 20% at 20°C.

2 Inoculum preparation

The microorganisms were inoculated in LB-Agar medium and incubated at 37 ° C from 12 to 24 hours.

3 Three colonies of the strains previously incubated were dissolved in 990 µL of saline solution (0.85% of NaCl).

4 The microbial suspension was adjusted to a concentration of $1X10^8$ CFU equivalent to 0.5 on the Mcfarland scale and an absorbance of 0.1 or 0.12 (*albicans*) at 600nm.

5 In the case of not reaching the necessary microbial concentration, one colony of the microorganism was added to the suspension. In case of exceeding the required microbial concentration, a serial dilution was performed with saline solution. In both cases read at 600 nm

6 **Disk diffusion**

The inoculum was diluted with saline solution to a final concentration of $1X10^6$

7 The inoculum was spread over the plates, leaving the inoculum to settle on the plate for 5 min.

8 A sterile paper disk was placed on the plates and deposited with the samples to evaluate in a volume range of 10-100 µL.

9 Sterile water and Ampicillin were used as controls. The plates were incubated at 37 °C for 24 hours. A transparent ring around the paper disk indicated antibacterial activity and the halo was measured.

10 **Microdilution assay**

250 µL of Mueller Hinton 2X medium was mixed with different concentrations of the extracts in the range of 50 to 170 µL and 50 µL of inoculum, adding sterile water until 500 µL of total solution.

11 A control was required for each mixture (without inoculum)

12 All samples were incubated at 37 °C for 12 h and the optical density (OD) at 600 nm was measured to determine the growth of microorganism. The assay was repeated 5 times per mixture