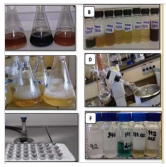

Feb 04, 2021

EXTRACTS PRODUCTION AND FRACTIONATION

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1 *Works for me* dx.doi.org/10.17504/protocols.io.br2cm8aw

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DOI

dx.doi.org/10.17504/protocols.io.br2cm8aw

PROTOCOL CITATION

Rene Flores Clavo, Cristian Daniel Asmat Ortega, Nataly Ruiz Quinones 2021. EXTRACTS PRODUCTION AND FRACTIONATION. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.br2cm8aw

KEYWORDS

EXTRACTS PRODUCTION, FRACTIONATION OF EXTRACTS, METABOLITES, BIOPROCESS

CREATED

Feb 02, 2021

LAST MODIFIED

Feb 04, 2021

PROTOCOL INTEGER ID

46884

MATERIALS TEXT

Materials

Erlenmeyer 2 L
pH meter
Notebook
Polypropylene tubes of 15 mL
Round bottom flask
Beakers 50 mL
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Nitrile gloves
Glass flask de 50 and 100 mL
C18 chromatographic column for Solid Phase Extraction (SPE), carbon content 9%, Specific surface 280 m2 / g, particle size 40-75µm, capacity 6mL Ref: Thermo Scientific ^{TM}

Reagents

Ethyl acetate (EtOAc)
Methanol (MeOH)
water (H_2O)
Glacial acetic acid 1%
Ethanol (EtOH)

Solutions

R2A broth (Difco ref. 2 34000)
Artificial Seawater (ASW) (0.1 g of (KBr) potassium bromide; 70 g of (NaCl) sodium chloride; 10.61 g of ($MgCl_2$-$6H_2O$) magnesium chloride; 1.47 g of ($CaCl_2$-$2H_2O$) calcium chloride; 0.66 g of (KCl) potassium chloride; 0.04 g of ($SrCl^2$ _ $6H_2O$) strontium chloride; 3.92 g of (Na_2SO4) sodium sulfate; 0.19 g of ($NaHCO_3$) sodium bicarbonate; 0.03 g of (H_3BO_3) boric acid)
NaCl
Glycerol 20%

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Analytical balance
Freezer
Rotary evaporator (R-215 Buchi)
Centrifugate
Water bath
Ultra turrax basic (IKA ref.02H2063.08.CC)
Shaker
Separatory funnel
Vacuum

DISCLAIMER:

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Pre-inoculum

1 Put three to four colonies of the bacteria in 5.0 mL of R2A broth supplemented with ASW with NaCl 7%, pH 7.0, and incubated at 28 °C for 5 to 7 days

Inoculum

2 After cultures grow, transfer the total volume to an Erlenmeyer of 500.0 mL containing the same culture broth and incubated for 7 days at 28 °C under agitation at 150 RPM followed by 23 days without agitation

3 Continue with the bioprocess for 28 days without agitation, maintaining incubation conditions.

Extraction

4 Add Ethyl acetate in culture broth in proportion 1:1 v/v in a beaker of 2 L. Rupture the cells in ultra turrax basic (IKA ref.02H2063.08.CC) at 7000 rpm for 15 min. and leave to macerate overnight.

5 Put the mix in a separatory funnel of 2 L, and recover the organic fraction

6 Add again ethyl acetate in the same proportion. Repeat steps 4 and 5 two times, without macerating overnight.

7 Obtain the organic fraction and concentrate the crude extract in a round bottom flask of 1L in the rotary evaporator (R-215 Buchi) under vacuum 200 mbar and temperature of 40 °C until the solvent is completely dried and store it at 4°C

8 Determine the dry weight of the obtained crude extract.

Fractionation

9 Fractionate the crude extracts of the isolates using a vacuum C18 chromatographic column for Solid Phase Extraction (SPE).

10 Solubilize the initial crude extract in methanol (6,4 mg/mL w/v).

11 Obtain the following fractions: 1 water (H_2O); fraction 2 water: methanol (H_2O: MeOH 1:1 v/v); fraction 3 methanol (MeOH); fraction 4 MeOH: ethyl acetate (EtOAc) (1:1 v/v); fraction 5 EtOAc 100%; fraction 6 EtOAc: Glacial acetic acid 1%

12 Dry these fractions in a rotary evaporator at 45 °C under vacuum 200 mbar, weight it and use it for further activities

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