Ministry of Higher Education and Scientific Research

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College of Education

Biology Department

Isolation and Identification of *Nocardia* spp. from Soil emphasizing on Development of Highly Producing Antimicrobial and Antitumor Strains

A Thesis

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Biotechnology

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Summary

One hundred and sixty nine *Nocardia* isolates were recovered from 111 samples from Iraqi soils by paraffin baiting and dilution techniques on six cultivation media including Glucose-asparagine agar, Sabouraud dextrose agar, Glycerol agar ,Nutrient agar , Modified Czapeks agar and Yeast extract agar. The range of physical and chemical parameters of soil samples were determined including temperature (21- 49.6°C),pH(7.1-8.1), salinity (1.03- 53.27 ppt) and calcium carbonate (0.65-12.22 mg/L).

The paraffin baiting technique was more efficient in the isolation of *Nocardia* than dilution technique and GAA medium was more suitable for cultivation of *Nocardia* isolates followed by SDA medium. All recovered isolates were submitted to primary screening of antibacterial activity on the basis of diameters of inhibition zones against the test organism *Staphylococcus aureus* NCTC6571(16-55mm) and *Escherichia coli* ATCC 25922 (16-38mm). The soil from north of Iraq was the richest in its content of *Nocardia* isolates.

Eleven isolates with the highest antimicrobial activities were selected for chemotaxonomic studies revealing the following : *N* .sp.1, *N.*sp. 2 ,*N* . sp. 3 , *N.* sp.4 , *N.* sp.5, *N.*sp. 6 , *N.* sp.7 , *N.* sp.8 , *N.* sp.9 , *N.* sp. 10 and *N.* sp. 11 which had mycolic acid with (R_f 0.9) .

*N.*sp.6 was chosen out for extraction processes due to its highest inhibition zone (mm) against different standard and clinical species of bacteria and yeasts during the secondary screening .

Test for the physiochemical properties of extract such as Gas Chromatography/Mass spectroscopy and Thin layer chromatography indicated that this extract was composed of 12 compounds namely beta Sitosterol, *o*-hydroxycinnamic acid, Eugenol, Alfa ethynylbenzyl alcohol , 3H-Pyrazol-3-one,2,4-dihydro-4,4,5-trimethyl , 1-(3-Hydroxy-4-methylphenyl)-1,3,3,6-tetramethylindan-5-o1, 1-pentanol-2-methyl, 3-aminophenol, 4-pyridinol, 3,5-bis(1,1-dimethyl)-1,2-Benzenediol, 1-(2-Hydroxy-4,6-dimethoxyphenyl)but-2-en-1-on and Glycidol.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against eight standard and clinical isolates of bacteria and yeasts . The cytotoxicity effect on human red blood cells of the extract was studied and the median lethal dose (LD50) for laboratory mice showed that it was not toxic in all concentrations studied (8 , 10 , 14 , 16 , 18 , 20 , 24 , 40 , 60 , 80 , 140 , 200, 500, 1000, 10000, 20000 mg/Kg).The extract also showed antitumor activity against L20B mouse cell lines with highest inhibition rate of 77.1% in the concentration 6.25 mg/mL.

The production of the extract from wild isolate *N.*sp.6 0.74 g/L was developed for maximum production by mutation with gamma radiation for different periods of time(1,5,15,30,45 min.) and with nitrosoguanidine at different concentrations (100,200,300,400,500 µg/L).This led to increase in production to 4.05 g/L and 20.8 g/L respectively.

Improvement of fermentation medium by the addition of either 1%paraffin, 0.05%K_2HPO_4, 2.5%,3% glycerol, 2% glucose or 2%sucrose increased production to 8.68, 7.46, 4.26, 1.13, 5.13 and 1.33g/L respectively by *N.*sp.6.

The extract production from mutated strains *N.*sp.6G and *N.*sp.6GN growing in improvement fermentation medium was calculated . Growing of *N.*sp.6G in fermentation medium with 1% paraffin and 0.05% K_2HPO_4 increase the production to19.5 and 31.7 g/L respectively but growing of *N.*sp.6GN in fermentation medium with1% paraffin and0.05% K_2HPO_4 decreased the production to 14.6 and 17.4 g/L respectively .