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Study the Antibacterial Activity of Alcoholic Extract of *Syzygium Aromaticum* on *Pseudomonas Aeruginosa* and Compare with Some Antibiotics

Luma Saeed Mohammed

University of Baghdad, College of Science, Biology Department

Abstract:

Forty *Pseudomonas aeroginosa* isolates isolated from Baghdad hospitals from different clinical specimens identified by morphology and biochemical tests then confirmed by vitek system . Different antibiotics used against isolates like ampicillin ,levofloxacin ,gentamycin , meropenem and ceftazidim ,the results showed that there were 100% of bacterial resistance to ampicillin, and gentamycin while 50% of resistance to ceftazidim , 10% of isolates had resistance to levofloxacin and meropenem. Minimal inhibitory concentration for alcoholic extract of *Syzygium aromaticum* was prepared and used against bacterial isolates, it had inhibition effect on bacteria in 200 mg/ml , and 100 mg/ml gave diameter of inhibition zone rang 12-18mm and 4-6mm respectively. Minimum inhibitory concentrations of plant extract were 200-100 mg/ml . *Syzygium aromaticum* extract had agood antibacterial activity on *P. aeruginosa* .

Key words

Antibiotcs resistant, *P.aeruginosa* , *Syzygium aromaticum*

Introduction:

P. aeruginosa the Gram negative bacterium that found in nature. It may be isolated from water, soil, plants and the general environment (1) . *P.aeruginosa* is a major bacterial pathogen in patients with cystic fibrosis (CF) (2). It is opportunisticc nosocomial pathogen, causes different and cause death in immuno-compromised patients (3). *P. aeruginosa* is a hospital acquired pneumonia , especially in patients in intensive care units (ICUs) n (4). *P.aeruginosa* resist to antibiotics, like aminoglycosides, quinolones and β-lactams , the major mechanisms of *P. aeruginosa* resistance used may be intrinsic and acquired resistance (5). The low outer membrane permeability, expression of efflux pumps that pumping out antibiotics out of the cell and the production of antibiotic-inactivating enzymes are intrinsic resistance .While the acquired resistance either horizontal transfer of resistance genes or mutational changes (6). The adaptive resistance of *P. aeruginosa* involves formation of biofilm in the lungs of infected patients where the biofilm serves as a diffusion barrier to reduce antibiotic penetration to the bacterial cells (7). In addition, multidrug-tolerant persister cells that are able to survive antibiotic attack can form in the biofilm ; these cells are responsible for prolonged and recurrent infections in CF patients. Interestingly, commercially used for many medicinal purposes and in the perfume industry, and clove is considered one of the spices that can be used as preservatives in many foods, especially

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in meat processing, to replace chemicals as a result their antioxidant and antimicrobial properties (8) .

Methods:

Bacterial isolation: two hundred samples (urine and sputum) were collected from patients from some Baghdad hospitals .

Identification of bacteria : bacteria identified morphologically and biochemically by hemolysin , pigments , Gram stain, shape under microscope , catalase , oxidase ,IMVIC tests (Indole,Meyhyl red and Voges-Proskauer ,citrate tests) urase and coagulase (9) , the identification was confirmed with vitek 2 compact system.

Antibiotics sensitivity tests:

Disc diffusion method (Kirby-Bauer method) was made (10), to colonies from 18 h. culture of *P.aeruginosa* were transferred to test tube contain 5 ml of normal saline to get 1×10^8 CFU/1 ml culture by compare with 0.5 McFarland tube. The procedure was as follow:

1- Inoculated the plates with a sterile swab .
2- Left the plates for a few minutes .
3- Antibiotic discs and E-test strips were placed on the inoculated plate by using a sterile forceps.
4- Incubation for 18-24 hrs. at 37 C 0 .
5- Reading the results for discs:after incubation, the diameter of each inhibition zone was measured in millimeter .
6- Read the MIC value of E-test. (11).

Preparation of plant extract and Soxhlet Extraction:

Dried powder of ***S. aromaticum*** was used in this study;25.0g of dried plant powder was extracted with 200 ml of 80% (v/v) aqueous ethanol by soxhlet extractor for 8 hrs, then the extract was dried by oven with 40 C 0 , the powder of extract kept in 4 C ^{0} (12).

Antibacterial activity of S. *aromaticum* :

Antimicrobial activity of S. *aromaticum* extract was proved by well method with using of Mueller-Hinton agar plates . Isolated bacteria were spreading on the surface of agar . 8 mm well were made in agar plates and extract were prepared as (200, 100, 50 and 25) mg/ml then put in wells. After incubation antibacterial activity was estimated by scaling the diameter of the inhibition zone around the wells (13) .

Determination of MIC of plant extract:

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MIC of crude extracts was determined with dilution method (macro-dilution). Set of Muller-Hinton broth tubes with (200, 100, 50 and 25) mg/ml of plant extract , inoculated by bacteria. After incubation, tubes were examined in variation in turbidity (indicator of growth). The first clear tube considered as MIC of S. *aromaticum* extract, against *P.aeruginosa*, positive and negative control were used for comparison.

Results and Discussion:

Isolation and identification of bacteria:

Two hundred urine samples from patients collected from some Baghdad hospitals from during 2020 . Forty samples were gave growth for bacteria .

Bacterial identification by biochemical tests:

Bacterial isolates were cultured on MacConkey agar, the result of morphological and biochemical tests of *P.aeruginosa* showed that *P.aeroginosa* isolates were Gram negative rods, positive tests to catalase , oxidase , Citrate , nitrate reduction and gelatin hydrolysis , while gave negative tests for MR,VP,indole and urease , forty isolate related to *P.aeruginosa* .

Antibacterial sensitivity test:

The results showed that there were 100% of bacterial isolates had resistance to ampicillin, and gentamycin while 50% of isolates gave resistance to ceftazidim , 10% of isolates had resistance to levofloxacin and meropenem. Other studies reported that there was 100% of resistance of *P.aeruginosa* resist ampicillin and other antibiotics such as penicillin ,cloxacillin, tetracycline and others (14). In this study levofloxacin and meropenem had a good activity on isolates (10% of resistance) , while in other study there was 75.3% of resistance of *P.aeruginosa* to levofloxacin and ciprofloxacin (15). Levofloxacin is a third generation fluoroquinolone antibacterial agent with a broad spectrum activity against Gram-positive and Gram-negative bacteria and atypical pathogens. The bactericidal activity by inhibiting topoisomerase IV and DNA gyrase (16).Meropenem is a parenteral carbapenem antibiotic has bactericidal activity against almost all clinically significant aerobes and anaerobes. Its high activity by ease of entry into bacteria combined with good affinity for essential penicillin binding proteins, including those associated with cell lysis, Breadth of spectrum is due, in part, to stability to all serine-based β-lactamases, including those which hydrolyse third-generation cephalosporins. Meropenem has an antibacterial spectrum which is broadly similar to that of lmipenem but, whilst slightly less active against staphylococci and enterococci, it is more active against *P.aeruginosa*, all Enterobactenaceae and *Haemoplulus influenzue* (17).

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Antibacterial activity of *S.aromaticum*:

Results showed an antibacterial effect of plant extract against *P.aeruginosa* at 200 mg/ml, and 100 mg/ml concentrations the diameter of inhibition zone rang 12- 15mm and 4-6mm at concentrations 200mg/ml and 100mg/ml respectively, from these results the effect of plant extract reduce in low concentrations , according to results, *S.aromaticum* extract with ethanol (80%) at concentrations (200, 100 , 50 25) mg/ml gave inhibition activity against *P.aeruginosa* but in low concentrations did not gave any activity. Other study reported that *S.aromaticum* had an inhibition effect on pathogenic bacteria ,*S.aromaticum* acts as anti-fungal ,anti-inflammatory and anti-microbial immunemodu-lator), anti-carcinogenic and anti-mutagenic (18). Medicinal plant parts (roots, leaves, branches/stems, barks, flowers and fruits) are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins(19).

Conclusion:

The plant extracts which proved to be potentially effective as (*P. aeruginosa*) can be used as natural alternative preventives to control bacterial diseases to avoid healthy hazards of chemically antimicrobial agent applications.

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