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Inhibitory effectiveness of bacterial alcoholic extracts against antibiotic-resistantand biofilm-producing bacteria isolated from differentinfections in a local environment

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ABSTRACT

This study evaluated the inhibitory effectiveness of organic solvent (ethanol, chloroform, and ethyl acetate) extracts of secondary metabolites from bacteria (*Bacillus subtilis*, *Kytococcus sedentarius*, and *Pseudomonas fluorescens*) isolated from the local environment in the city of Ramadi against bacterial growth. The diffusion method was used to investigate the susceptibility of the isolates to 10 different multidrug-resistant bacteria and biofilm producers isolated from clinical samples (urinary tract infections, diarrhea, wound infections, and burns). Alcoholic extracts have shown antibacterial activity against a wide range of bacteria, including Gram-positive and Gram-negative bacteria. Results showed that the ethanol extract of *P. fluorescens* highly inhibited the growth of *Staphylococcus aureus* with an inhibition zone diameter of 23±2 mm. Meanwhile, it was less effective against *Escherichia coli* with an inhibition diameter of 10±2 mm. The chloroform extract of *K. sedentarius* had the highest activity against *Acinetobacter baumanii* with an inhibition zone diameter of 28±2 mm. The ethyl acetate extract of *B. subtilis* highly inhibited the growth of *S. aureus* with an inhibition zone diameter of 31±2 mm and had the lowest effectiveness against *Klebsiella pneumonia* with an inhibition diameter of 20±2 mm. The ethyl acetate extract of *P. fluorescens* had the highest inhibition against *S. aureus* bacteria (31±2 mm) and the lowest effectiveness against *Pseudomonas. aeruginosa* (9±1 mm).

1. INTRODUCTION

Numerous manufactured chemical pharmaceuticals can fight microorganisms, but many of them have side effects. Furthermore, their prolonged usage can lead to their loss of effectiveness and the development of resistance in germs [10,11].

Despite the unprecedented development in the medical domain, many infectious diseases still pose a threat to human health through the emergence of many resistant strains resulting from the indiscriminate use of antibiotics [5]. The lack of efficiency of medical drugs has led to great interest in plants and natural products through the utilization of secondary metabolites as therapeutic alternatives, raw materials for medicinal drug production, or a source of active substances in medicine formulation [16].

Since ancient times, humans have relied on primitive treatment methods, using materials in their raw form, such as plant or animal materials, which are effective against pathogenic microorganisms [12]. Antibiotic-producing microorganisms are naturally widespread and found in soil, water, and the decaying residuals of plants and animals. Soil is the primary source for their isolates. Therefore, many researchers focused on the soil to obtain microorganism strains for producing new antibiotics [14]. Hence, the idea of using nonpathogenic microorganisms to inhibit the pathogenic ones present in cultivated soil without affecting the rest of the microbial groups has been initiated [6,7]. Bacteria produce secondary metabolic compounds through a series of chemical reactions, which start from the intermediate compounds produced during basic metabolic processes, such as the metabolism of carbohydrates and proteins.

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Scientific evidence has denoted the efficiency of biotherapeutics in influencing pathogens, and this approach includes many types of bacteria [1]. The term antibiotics were used for the first time in 1942 by the scientist Waksman, who defined antibiotics as metabolic substances produced by microorganisms that inhibit the growth of other microorganisms without affecting the producing bacteria [9]. Secondary metabolites are a wide range of chemical compounds produced by living organisms, including bacteria. They are usually generated as side products or byproducts of primary metabolism, breaking down organic compounds to produce energy. In some cases, secondary metabolites are produced specifically for a particular purpose, such as defending the organism against predators or competitors. Humans have also used secondary metabolites in various applications, including the pharmaceutical industry where many secondary metabolites have been used as drugs or drug ingredients. Many physical and chemical methods have been tried to overcome antibiotic resistance and biofilm production [19]. However, the materials used are expensive, require a long period to show their effect, or decompose to produce environmentally toxic substances. For this reason, researchers have searched for naturally sourced materials that are environmentally friendly, low cost, and have low or no toxicity, such as microorganisms. Bacteria produce antibiotics in various environments, including soil, water, and food. Many of the first antibiotics were found in natural environments, such as soil or water. Several antibiotics have been discovered through research and development, such as using bacteria species (*Bacillus subtilis, Kytococcus sedentarius*, and *Pseudomonas fluorescens*), which are widespread in soil and water, have simple growth requirements, and can produce a wide range of non- or low toxic substances that are effective against other microorganisms and can withstand extreme environmental conditions, such as high or low temperature or pH. These characteristics made them the focus of research on utilizing these bacteria or their secondary metabolites for inhibiting the growth of antibiotic-resistant bacteria or biofilm producers [23]. This study aimed to investigate the effect of organic solvent extracts (ethanol, chloroform, and ethyl acetate) from bacteria (*B. subtilis, K. sedentarius*, and *P. fluorescens*) isolated from local environments, extract their secondary metabolites, and examined their inhibitory ability against antibiotic-resistant bacteria and biofilm producers.

2. MATERIALS AND METHODS

2-1. Bacterial isolates

Previously identified environmental bacterial isolates were obtained from the laboratory of the Department of Biology, College of Education for Pure Sciences, University of Anbar by the department researchers. Three bacterial isolates (*B. subtilis* and *K. sedentarius* from soil and *P. fluorescens* from water) were obtained. Samples were also collected from patients suffering from urinary tract infections, diarrhea, and wound and burn infections from 1/10/2022 to 1/2/2023. Bacterial species were isolated from the samples collected in the Women and Children's Hospital and Ramadi General Hospital.

2-2. Bacteria identification

The isolates were identified through the following morphological and biochemical tests and based on scientific sources used internationally for bacterial identification: microscopic examination and cultural traits including the IMViC tests (indole test, methyl red test, Voges–Proskauer test, and citrate consumption test), catalase test, and oxidase test. Assessment for some virulence factors included tests of urease, protease degradation, hemolysis, antibiotic resistance, and biofilm formation. The samples consisted of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis,* and *Acinetobacter baumanii*.

2-3. Production of virulence factors

The ability of the isolates (*S. aureus, P. aeruginosa, K. pneumoniae, E. coli, P. mirabilis,* and *A. baumanii*) from different infections to produce four virulence factors (hemolysin enzyme production, urea enzyme production, protease enzyme production, and biofilm formation) was tested.

2-4. Testing the susceptibility of the bacterial isolates

to antibiotics

The susceptibility of bacteria to the following antibiotics was tested following the Kirby–Bauer procedure [32]: amoxicillin clavulanic, piperacillin, ceftriaxone, ceftazidime, cefotaxime, amikacin, meropenem, imipenem, ciprofloxacin, and levofloxacin.

2-5 Biofilms production

Biofilm formation was detected with the sterile microtiter plate method of 96 wells [13].

2-6 Production of secondary metabolic compounds by bacterial isolates

Isolates from *B. subtilis, K. sedentarius, and P. fluorescens* were grown in liquid SOY media (prepared in the laboratory) [20] and incubated in a shaking incubator at a speed of 120 rpm and a temperature of 30 °C for 72 hours. The bacterial cultures were then centrifuged at a speed of 10,000 rpm for 20 minutes, and the supernatant was sterilized by passing it through a 0.22 mm filter paper. The bacterial activity was tested against Gram-positive bacteria, represented by MRSA, and Gram-negative bacteria, represented by *P. aeruginosa, E. coli, P. mirabilis, A. baumanii,* and *K. pneumoniae*, by using the method of wells [20].

2-7 Secondary metabolite extraction from the bacterial isolates

The secondary metabolites of bacteria (*B. subtilis, K. sedentarius, and P. fluorescens*) were extracted as previously described [28]. The product was dissolved in three organic solvents of different polarities, namely, ethanol (99.9), chloroform (99) as a polar solvent, and ethyl acetate (88.11) as a medium polar solvent. After 100 ml of the filtrate was mixed with 100 ml of each solvent, the mixture was left in a water bath for 24 hours. The extracted material was concentrated in a rotary evaporator at a temperature of 40 °C–45 °C. According to the concentrations in the experiment, a stock solution of the organic solvent extract was prepared to test the inhibitory effectiveness against the tested bacteria.

2-8. Bacterial dilution preparation

The bacterial dilution was prepared as previously described [2] by growing the activated bacteria on nutrient agar media and incubating them at 37 °C for 24 hours. Ten colonies of each bacteria used in the experiment were transferred to a tube containing 5 ml of nutrient broth media under sterile conditions and then incubated at 37 °C for 4–6 hours. Appropriate dilutions were made for each type of bacteria to achieve a cell count of approximately 10 per ml.

2-9 Antagonistic effectiveness test of bacterial extracts

The susceptibility of pathogenic bacteria to the bacterial extracts was tested using the method in [3], where the dishes containing the Mueller–Hinton agar were inoculated by spreading 0.1 ml of the bacterial suspension. The plates were left at room temperature for 15 minutes to absorb the vaccine, after which wells were made in the nutrient media inoculated with bacteria using an 8 mm diameter sterile perforator. A micropipette was used to transfer 50 µl of each concentration of the bacterial extracts to each well. Control dishes were also prepared by placing 50 µl of organic solvent in the well instead of the bacterial extract and then incubated at 37 °C for 18–24 hours. Finally, a ruler was used to measure the inhibition zone diameter, representing the area of nongrowing bacteria surrounding the well.

2-10. Secondary metabolite production

Isolates of *B. subtilis, K. sedentarius*, and *P. fluorescens* were cultured in liquid SOY media (prepared in the laboratory) and incubated in a shaking incubator at a speed of 120 rpm and a temperature of 30 °C for 72 hours. The highest production of secondary metabolites was recorded for *Bacillus* spp. 48 hours after growth when the bacterial culture entered the idiophase of the stationary phase. The production of secondary metabolites begins when the bacterial growth rate decreases as a result of the consumption of one of the major nutrients, such as carbon, nitrogen, or phosphorus [27].

2-11. Test of the effectiveness of the bacterial supernatant against the target bacteria

The ability of the microorganisms isolated from local environments to inhibit pathogenic bacteria was tested. After the bacteria were grown on soy media, they were

placed in test tubes and centrifuged at 10,000 rpm for 20 minutes. The resulting supernatant was separated with 0.22 filter paper to ensure no bacterial cells had passed. The effectiveness of the obtained filtrate containing secondary metabolites was tested by spreading one of the test bacteria in Mueller–Hinton agar and leaving it for 15 minutes. The filtrated supernatant was placed in a well and incubated at 37 °C for 24 hours. The antibiotic-producing microbes were detected through the appearance of inhibition zones around the well [4].

2-12.Extraction of bacterial secondary metabolites with organic solvents

The secondary metabolites of *B. subtilis*, *K. sedentarius*, and *P. fluorescens* bacteria were extracted as previously described [28]. The product was dissolved with organic solvents (ethanol, chloroform, and ethyl acetate) in a ratio of 50:50.

2-13. Statistical analysis

The experimental results were designed using the SPSS program for data analysis to study the effect of different treatments on the studied traits.

3. RESULTS AND DISCUSSION

3-1. Bacteria isolation and identification

Isolation and identification through microscopic and biochemical tests showed that Gram-negative bacteria and Gram-positive bacteria accounted for 77.83% and 22.17% of the total number of infections, respectively, as confirmed using the VITEK 2 System for the species (*S. aureus, P. aeruginosa, K. pneumoniae, P. mirabilis, A. baumannii, and E. coli*). The higher number of Gram-negative bacteria isolates compared with Gram-positive bacteria isolates in infections is due to their increased resistance to antibiotics and their virulence factors, such as endotoxins that are present within the outer cells' walls and released after the germs enter the bloodstream [17]. hemolysin enzyme production, urea enzyme production, protease enzyme production, and biofilm formation

3-2. Virulence factor production

3-2. Virulence factor production

The ability of common bacteria isolated from different infections to produce the following four virulence factors was tested: hemolysins enzyme production, urease enzyme production, protease enzyme production, and biofilm formation. The results were as follows.

Table 1. Bacterial isolates' ability to produce virulence factors

Isolate	Hemolysins production	Urease	Protease	Biofilm formation
S.aureus no(43)	**100%**	**70%**	**0%**	**87.5%**
E. coli no(52)	**65%**	**0%**	**100%**	**90.9%**
P. mirabilis no(15)	**100%**	**100%**	**100%**	**100%**
K.pneumoniae no(28)	**0%**	**100%**	**0%**	**88.8%**
Ps.aeruginosa no(42)	**100%**	**100%**	**100%**	**90.9%**
A. bumannii no(13)	**0%**	**0%**	**100%**	**100%**

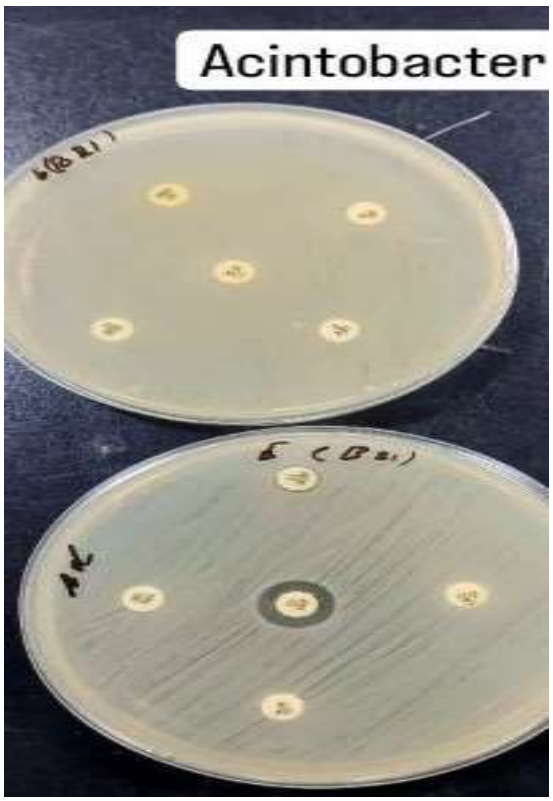
3-3. Antibiotic susceptibility test

The antibiotic resistance of common bacteria isolated from different infections was also studied. Some *S. aureus* isolates showed resistance to various antibiotics (piperacillin, amoxiclav, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, amikacin, imipenem, and meropenem by 100%, 98%, 96%, 78%, 100%, 67%, 89%, 80%, 90%, and 88%, respectively) and susceptibility to others. These findings agreed with previous studies [33, 41]. *P. aeruginosa* isolates showed 100% resistance to amoxiclav and piperacillin, which are part of the penicillin group, which was consistent with [18]. For ceftriaxone, cefotaxime, and ceftazidime, which are part of the cephalosporins group, the resistance percentage was also 100% [34]. The resistance to amikacin, imipenem, meropenem, ciprofloxacin, and levofloxacin was 85%, 45%, 28%, 30%, and 32%, respectively, agreeing with the results of [35,36]. *K. pneumoniae* was resistant to ceftriaxone, ceftazidime, amoxiclav, piperacillin, and cefotaxime by 83.68%, 94%, 100%, 98%, and 96%, respectively. These results were close to the findings of [44]. The resistance to amikacin, imipenem, meropenem, ciprofloxacin, and levofloxacin was 50%, 77%, 85%, 100%, and 86%, respectively, which was almost consistent with [45]. The resistance of *E. coli* to ceftazidime, cefotaxime, and ceftriaxone reached 92%, 93%, and 100%, respectively, and that to the penicillin antibiotics piperacillin and

amoxiclav was 95% and 98%, respectively. This result was consistent with [37] in India, which showed high susceptibility to imipenem and meropenem by 88% and 89%, respectively. [46] showed that susceptibility of *E. coli* isolates to imipenem and ciprofloxacin was 100% and 79%, respectively, which was consistent with [38]. For levofloxacin and amikacin, *E. coli* showed resistance percentages of 84% and 93%, respectively, which were consistent with [8]. The bacterial isolates of *A. baumannii* showed high resistance to all types of tested antibiotics (100%), which was consistent with [39,40]. *P. mirabilis* was resistant to ceftriaxone, piperacillin, ceftazidime, and cefotaxime by 100%, which was almost consistent with [42]. *P. mirabilis* bacteria had high resistance to amoxiclav, levofloxacin, and ciprofloxacin (100% and 98%). The resistance of *P. mirabilis* to imipenem and meropenem has reached 100%. For the antibiotics belonging to the aminoglycosidic group, the resistance to amikacin was 100%. This finding was consistent with [42], as their isolates were 100% resistant to the two antibiotics.

Table 2. Resistance percentage of the studied bacteria

Bacteria / Antibiotics	*E. coli* no 53	*S.aureus* no 43	*Ps.aeruginosa* no 42	*K.pneumoniae* no 28	*P. mirabilis* no 15	*A. bumannii* no 13
Amox-clav	(98%)53	(100%)43	(100%)42	(100%)28	(100%)15	(100%)13
Piperacillin	(95%)53	(93%)43	(100%)42	(98%)28	(100%)15	(100%)13
Cefotaxime	(93%)53	(78%)43	(100%)42	(96%)28	(100%)15	(100%)13
Ceftazidime	(92%)53	(96%)43	(100%)42	(94%)28	(100%)15	(100%)13
Ceftriaxone	(100%)53	(100%)43	(100%)42	(83.68%)28	(100%)15	(100%)13
Amikacin	(93%)53	(80%)43	(85%)42	(50%)28	(100%)15	(100%)13
Imipenem	(88%)53	(90%)43	(45%)42	(77%)28	(100%)15	(100%)13
Meropenem	(89%)53	(79%)43	(28%)42	(85%)28	(100%)15	(100%)13
Ciprofloxacin	(79%)53	(67%)43	(32%)42	(100%)28	(98%)15	(100%)13
Levofloxacin	(84%)53	(89%)43	(30%)42	(86%)28	(98%)15	(100%)13



Antibiotic Inhibition Zone Diameters Against Bacteria *A. baumanii*

Antibiotic Inhibition Zone Diameters Against Bacteria *ps.aeruginosa*

3-4. Bacteria identification

The isolates selected from the Laboratory of Biology in the College of Education for Science were distinguished through their effectiveness in producing secondary metabolites as *B. subtilis*, *K. sedentarius*, and *P. fluorescens*.

3-5. Extraction of bacterial secondary metabolites with organic solvents

The secondary metabolites of *B. subtilis* extracted with ethyl acetate had the best antagonistic efficiency against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *A. baumannii*, and *E. coli* compared with the metabolites extracted with the other organic solvents. Several solvents can be used to extract secondary metabolites from *B. subtilis* [25]. This study demonstrated the apparent inhibitory activity of *B. subtilis* secondary metabolites against Gram-negative and Gram-positive bacteria (*S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *A. baumannii*, and *E. coli*). This result is consistent with [26], which indicated that the secondary metabolites extracted from *P. fluorescens* with ethyl acetate exhibited the best noticeable antagonistic activity against the target bacteria compared with the metabolites extracted with other organic solvents. The researchers also found that the secondary metabolites extracted from *K. sedentarius* with ethyl acetate had significantly better antagonistic activity against the target bacteria than those extracted with other organic solvents. They suggested that the effectiveness of ethyl acetate in extracting secondary metabolites may be due to its ability to proteolysis and lipolysis, resulting in the release of secondary metabolites from bacterial cells [26].

3-6. Antibacterial inhibitory effectiveness of extracts determinedby well diffusion

Antagonism tests showed significant variation between extracts and between solvents. The antibacterial effectiveness of *B. subtilis* extracts against the antibiotic-resistant bacteria was determined through diffusion in wells by measuring the diameters of inhibition around the wells on the Mueller–Hinton agar. The ethyl acetate extract of *B. subtilis* had the highest inhibitory effectiveness against *S. aureus* with an inhibition zone diameter of 31±2 mm, followed by *E. coli* at 24±2 mm, *P. aeruginos*a at 23±2 mm, *A. baumanii* at 30±2 mm, *P. mirabilis* at 30±2 mm, and the lowest effectiveness on *K. pneumoniae* at 20±2 mm. For the chloroform extract, the greatest inhibitory effectiveness was observed for *S. aureus* with the largest inhibition zone diameter of 25±2 mm, followed by *E. coli* at 13±2 mm, *P. aeruginosa* at 15±2 mm, *A. baumanii* at 20±2 mm, *K. pneumoniae* at 12±2 mm, and the lowest effect on *P. mirabilis* at 11±2 mm. For the ethanol extract, the greatest effectiveness was recorded against S. *aureus* with the highest inhibition zone diameter of 16±2 mm, followed by *E. coli* at 15±2 mm, *P. aeruginosa* at 13 ±2 mm, *A. baumanii* at 15±2 mm, *K. pneumoniae* at 13±2 mm, and the lowest effect on *P. mirabilis* at 11±2 mm. *Bacillus* is a rich source of lipopeptides, organic acids, diacetyl, protease, lipase, hydrogen peroxide, amylase, ethanol,

bacteriocin, and other antibacterial substances that are produced during lactic fermentation [29]. The excellent protein secretion ability of *B. subtilis* makes it an essential host for producing proteins, including subtilosin A, a cyclic peptide produced by *B. subtilis* as one of its primary bacteriocins. The specific action of subtilosin A involves anchoring to a membrane receptor while electrically bound to the plasma membrane [21] this electrostatic binding dissipates the pH gradient across the membrane, causing an influx of intracellular ATP that starves the cell and ultimately leads to its death. Bacteriocins isolated from bacteria are a valuable alternative to antimicrobial agents for combating infections. They have shown antimicrobial activity against several bacteria, including Gram-positive, Gram-negative, aerobic, and anaerobic bacteria [31]. Therefore, *B. subtilis* shows potential as a new therapeutic agent. This result is similar to the findings in [30], which indicated that *B. subtilis* had antibacterial activity against *E. coli* isolates with 18 mm growth inhibition zone at the dilution of 0.01 µl/ml. [22] also showed that *B. subtilis* metabolites inhibited *P. aeruginosa, S. aureus, E. coli, P. mirabilis, K. pneumoniae, Salmonella typhi, Bacillus cereus, Corynebacterium diphtheria,* and *Dysentriae shigella*).

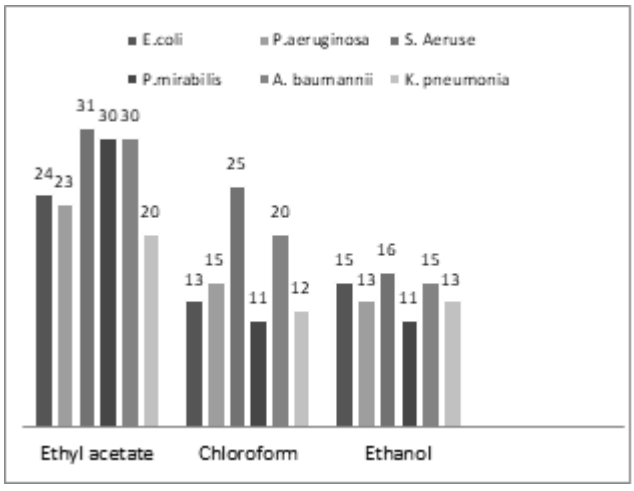


Fig1. Effect of *B. subtilis* extract with organic solvents (ethanol, chloroform, and ethyl acetate) against antibiotic-resistant bacteria

The antibacterial effectiveness of *K. sedentarius* extracts against antibiotic-resistant bacteria was studied using the method of diffusion at the wells by measuring the diameters of inhibition around the wells on the Muller–Hinton agar. The ethyl acetate extract of *K. sedentarius* exhibited the greatest effectiveness on *S. aureus*, producing the highest inhibition zone diameter of 24±2 mm, followed by *E. coli* at 20±2 mm, *P. aeruginosa* at 18±2 mm, *A. baumanii* at 30±2 mm, *K. pneumoniae* at 21±2 mm, and the lowest effect on *P. mirabilis* at 15±2 mm. The ethanol solvent extract of *K. sedentarius* also showed inhibitory effectiveness against antibiotic-resistant bacteria, exhibiting the highest effectiveness on *S. aureus* with the highest inhibition zone diameter of 19±2 mm, followed by *E. coli* at 11±2 mm, *P. aeruginosa* at 15±2 mm, *A. baumanii* at 17±2 mm, *P. mirabilis* at 13±2 mm, and the lowest effect on *K. pneumoniae* at 10±2 mm. For the chloroform extract, the greatest inhibitory effectiveness was observed on *S. aureus* with the largest inhibition zone diameter of 24±2 mm, followed by *E. coli* at 20±2 mm, *P. aeruginosa* at 18±2 mm, *A. baumanii* at 30±2 mm, *K. pneumoniae* at 21±2 mm, and the lowest on *P. mirabilis* at 13±2 mm.

Through the method of diffused wells, the inhibitory activity was determined by measuring the inhibition zone diameter in the Muller–Hinton agar. The ethyl acetate extract of *P. fluorescens* had inhibitory effectiveness against the antibiotic-resistant bacteria, with the highest inhibitory area of 33±2 mm on *S. aureus*, followed by 22±2 mm on *P. mirabilis*, 30±2 mm on *P. aeruginosa*, 30±2 mm on *A. baumanii*, 20±2 mm on *K. pneumoniae*, and the lowest of 13±2 mm on *E. coli*. The results also revealed the inhibitory effectiveness of the ethanol extract of *P. fluorescens* against antibiotic-resistant bacteria, with the largest inhibition diameter of 25±2 mm on *S. aureus*, followed by 13±2 mm on *K. pneumoniae*, 11±2 on *P. aeruginosa*, 18±2 mm on *A. baumanii*, 15±2 mm on *P. mirabilis*, and the lowest of 10±2 mm on *E. coli*. The chloroform extract of *P. fluorescens* also showed inhibitory effectiveness against the antibiotic-resistant bacteria, with the highest effect on *S. aureus* with an inhibition diameter of 20±2 mm, followed by *E. coli* at 16±2 mm, *P. aeruginosa* at 15±2 mm, *A. baumanii* at 19±2 mm, and the lowest on *K. pneumoniae* at 12±2 mm. In [47], the growth of human pathogens such as *S. typhi, Shigella sp., Klebsiella sp., E. coli*, and *Staphylococcus*

ssp. were inhibited by all of the cultures of the cell-free supernatants from *P. fluorescens* isolates.

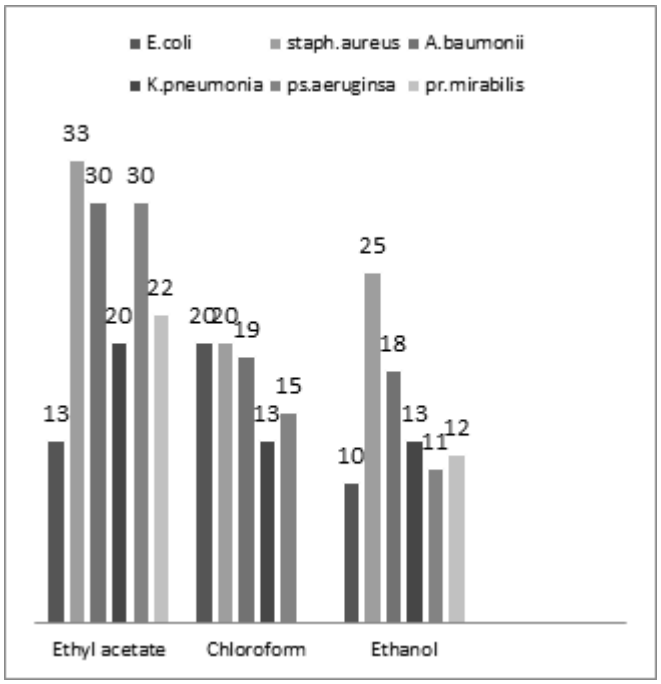


Fig2. Effect of *Kytococcus sedentarius* extract with organic solvents (ethanol, chloroform, and ethyl acetate) against antibiotic-resistant bacteria

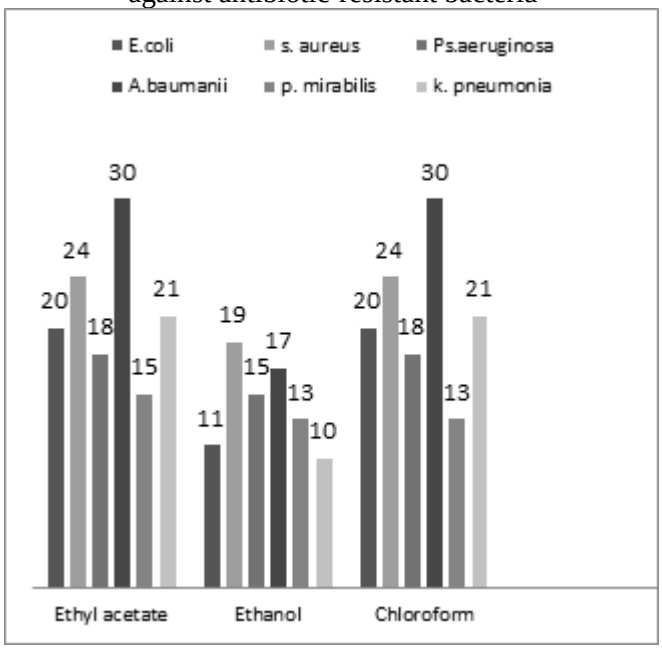


Fig3. Effect of *Ps. fluorescent* extract used with organic solvent(Ethanol, chloroform ,Ethyl acetate) against antibiotic-resistant bacteria

The antagonistic activity of the extracts was higher than that of the raw bacterial supernatant of the same isolate. This finding is consistent with [24], which inferred that the inhibitory activity of bacterial secondary metabolite extract is higher than that of the raw supernatant of the same bacteria cultured in liquid culture media. This phenomenon may be due to the low external permeability of Gram-negative bacteria and the presence of a lipopolysaccharide layer that acts a barrier against hydrophobic compounds.

Conclusions

Beneficial bacteria isolated from local environments can be an effective tool in combating pathogenic bacteria and can be used as a food supplement or as a preventive or therapeutic treatment for bacterial infections.

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References:

[1] .Al-Khafaji, Z. M. (2008), Microbiology, University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq, p. 77

[2] .Hussein, F. T. Q. (1981) Medicinal plants, their cultivation and components, dar-almareekh for publishing, Riyadh,.

[3] . Majeed, S. H. and Mahmoud, M. J. (1988) Iraqi plants and herbs between folk medicine and scientific research. First edition. Dar Al-Thawra for Printing and Publishing, Baghdad,.

[4] .Muhammad, A. and Abdul Latif K. (2022) Production of antibiotics from bacteria isolated from the marine environment, Journal of Biological and Medical Sciences 25.2 (2022): 31-38.

[5]. Pillai, S.P.; Pillai, C.A.; Shankel, D.M.; Mitscher, L.A.(2001).The ability of certain antimutagenic agent

to prevent development of antibiotic resistance. J.Muta.Res.,496,61-73.

[6] .Shaukat, S.S. and Siddiqui, I.A. 2003. The influence of mineral and carbon source on biological control of charcoal rot fungus Macrophomina phaseolina by fluorescent Pseudomonas in tomato. Lett. Appl. Microlbiol. 36: 392 – 398.

[7].Gram.L.; Melchiorsen, J.; Spanggaard, B.; Huber, I. and Nielsen. T. F. 1999. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. Appl. Environ. Microbiol. 65:969 – 973.

[8] .Paltansing, S. (2015). Antimicrobial Resistance In Enterobacteriaceae :Characterization and Detection . Leiden University. 155pp.

[9] .Waksman , S. A. (1967) . The actinomycetes: a summary of current knowledge. The Ronald press Co. New york .

[10] .Chan ED,Iseman MD(2008)Multidrug-resstant and extensively drug resistant tuberculosis:A reVivew.Curr.Opin.Infect.Dis.21:577-595.

[11] .Gandhi NR,Moil A, ,Sturm AW,Pawinski R,Govender T,Lalloo U ,Zeller K. Andrews isJ,Friedland G(2006)Extensively drug resistant tuberculosis a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa.Lancet.368:1575-1580.

[12] .Talaro , K. and Talaro , A. (1996) . Fundations in microbiology , basic principles , Times mirror higher education group , Wm . C. Brown Publishers . Chicago .

[13] .Fusco, A.; Coretti, L.; Savio, V.; Buommino, E.; Lembo, F. and Donnarumma, G. (2017). Biofilm Formation and Immunomodulatory Activity of Proteus mirabilis Clinically Isolated Strains. Int J Mol Sci.18(2): 1-11.

[14] .Egorov , N. S. (1985) . Antibiotics ascientific approach . Mir publishers , Moscow .

[15] .Su, Y.; Liu, C.; Fang, H. and Zhang, D. (2020). Bacillus subtilis: A universal cell factory for industry, agriculture, biomaterials and medicine. Microbial Cell Factories, 19(1), 1–12.

[16] .Gorlenko, C. L.; Kiselev, H. Y.; Budanova, E. V.; Zamyatnin, A. A.; and Ikryannikova, L. N. (2020). Plant Secondary Metabolites in the Battle of Drugs and Drug-Resistant Bacteria: New Heroes or Worse Clones of Antibiotics? Antibiotics, 9(4), 170.

[17] .Miller, L. S.; Fowler Jr, V. G.; Shukla, S. K.; Rose, W. E.; and Proctor, R. A. (2020). Development of a vaccine against Staphylococcus aureus invasive infections: Evidence based on human immunity, genetics and bacterial evasion mechanisms. FEMS Microbiology Reviews, 44(1), 123-153.

[18] .Al-Suwaidi, W. K. I. M. (2017). Diagnosis of Pseudomonas aeruginosa using multiplex PCR technique based on 16S rRNA, oprL, and oprD genes. College of Education for Pure Sciences, University of Anbar.

[19] .Ahmed A., Khan A.K., Anwar A., Ali S.A., and Shah M.R. (2016). Biofilm inhibitory effect of chlorhexidine conjugated gold nanoparticles against Klebsiella pneumoniae. Microbial Pathogenesis, 98: 50–56.

[20] .Dusane D.H., Pawar V.S., Nancharaiah Y.V., Venugopalan V.P., and Kumar A.R. (2011). Antibiofilm potential of a glycolipid biosurfactant produced by a tropical marine strain of Serratia marcescens. Biofouling, 27: 645- 654.

[21] .Noll, K.S.; Sinko, P.J. and Chikindas, M.L. (2011).Elucidation of the Molecular Mechanisms of Action of the Natural Antimicrobial Peptide Subtilosin against the bacterial Vaginosis-associated Pathogen Gardnerella vaginalis. Probiotics Antimicrob. Proteins, 3, 41-47.

[22] .Ozabor , T. P. and Fadahunsi, I. F. (2019). Antimicrobial Activity of Bacillus Subtilis Against Some Selected Food Borne Pathogens.Acta Scientific Microbiology (ISSN:2581-3226) .

[23] .Sawale A., Kadam T.A., and Karale M.A. (2014). Antimicrobial activity of secondary metabolites from halophilic Bacillus pumilus sp. Int. J. Current Microbiol. Appl.Sci.,3(6): 506-512.

[24] .Wilson G.S., Raftos D.A., and Nair S.V. (2011). Antimicrobial activity of surface attached marine bacteria in biofilms. Microbiol. Res., 166: 437-448.

[25] .Marhaeni B., Radjasa O.K., Khoeri M.M., Sabdono A., Bengen D.G., and Sudoyo H. (2011). Antifouling activity of bacterial symbionts of seagrasses against marine biofilmforming bacteria. J. Environ. Prot. (Irvine,. Calif)., 02: 1245–1249.

[26] .Mohan G., Kumar A., Thangappanpillai T., and Ramasamy B. (2016). Antimicrobial activities of secondary metabolites and phylogenetic study of sponge endosymbiotic bacteria, Bacillus sp. at Agatti Island Lakshadweep Archipelago. Biotechnol. Reports, 11: 44- 52.

[27] .Teasdale M., Liu J., Wallace J., and Rowley D.C. (2009). Secondary metabolites produced by a marine Halobacillus salinus that inhibit quorum sensing controlled phenotypes in Gram-negative bacteria. Appl. Environ. Microbiol., 75(3): 567-572.

[28] .Amin, N. T. D. A. (2017). Effect of aqueous and alcoholic extracts of garlic and ginger plants on some types of bacteria isolated from tonsillitis. College of Education for Girls, Tikrit University.

[29] .ZHANG X, AL-DOSSARY A, HUSSAIN M, SETLOW P & LI J. 2020. Applications of Bacillus subtilis Spores in Biotechnology and Advanced Materials. Appl Environ Microbiol 18 86(17): e01096-20.

[30] .Ali, Z. H. (2021). Synergetic effects of Bacillus subtilis as probiotics and Chitosan Nanoparticles against some Pathogenic Bacteria isolated from Burned Wound Infections. . M. Sc. Thesis. Faculty of Science. University of Kufa.

[31] .Tazehabadi, M. H.; Algburi, A.; Popov, I. V.; Ermakov, A. M.; Chistyakov, V. A.; Prazdnova, E. V; Weeks, R. and Chikindas, M. L. (2021). Probiotic Bacilli Inhibit Salmonella Biofilm Formation Without Killing Planktonic Cells. Frontiers in Microbiology, 12, 242.

[32] .Vandepitte, J.; Verhaegen, J.; Engbaek, K.; Rohner, P.; Piot, P. and Heuck, C. C. (2003). Bacteriological Investigations. In: World Health Organization .2nded. Basic Laboratory Procedures in Clinical Bacteriology, WHO, Geneva. 167pp .

[33] .Ismaeil, M. I. (2017). Antimicrobial Resistance Patterns and Extended Spectrum Beta-lactamases Producing by Proteus mirabilis Isolated from Different Sources. Al-Mustansiriyah Journal of Science, 28(1), 47-54pp. 758-76.

[34] .Rahimzadeh, M.; Habibi, M.; Bouzari, S. and Karam, M. R. A. (2020). First Study of Antimicrobial Activity of Ceftazidime-Avibactam and CeftolozaneTazobactam Against Pseudomonas aeruginosa Isolated from Patients with Urinary Tract Infection in Tehran, Iran. Infection and Drug Resistance, 13, 533.

[35] .Tumbarello, M.; Raffaelli, F.; Peghin, M.; Losito, A. R.; Chirico, L.; Giuliano, G. and Bassetti, M. (2020). Characterisation and risk-factor profiling of P. aeruginosa urinary tract infections: pinpointing those likely to be caused by multidrug-resistant strains. International Journal of Antimicrobial Agents, 105900.

[36] . Fabre, V.; Amoah, J.; Cosgrove, S. E. and Tamma, P. D. (2019). Antibiotic therapy for Pseudomonas aeruginosa bloodstream infections: how long is long enough?. Clinical Infectious Diseases, 69(11), 2011-2014.

[37] .Suresh, S., Karthikeyan, S., Saravanan, P., Jayamoorthy, K., Dhanalekshmi, KI. (2016). Comparison of antibacterial and antifungal activity of 5-amino-2- mercapto benzimidazole and functionalized Ag3O4 nanoparticles. Karbala International Journal of Moder Science, 2(2):129-137. doi:10. 1016/j. kijoms. 2016. 03. 003.

[38] . Tajbakhsh, E.; Ahmadi, P. Abedpour-Dehkordi, E.; Arbab-Soleimani, N. and Khamesipour, F. (2016). Biofilm Formation, Antimicrobial Susceptibility, Serogroups and Virulence Genes of Uropathogenic E. coli Isolated from Clinical Samples in Iran. Antimicrob Resist Infect Control. 5(11): 1-8.

[39] .Anwar, M.; Ejaz,H.; Zafar,A.; Hamid,H.(2016). Phenotypic Detection of Beta-Lactamases in Carbapenem Resistant Acinetobacter baumannii MetalloIsolated from Pediatric Patients in Pakistan http://dx.doi.org/10.1155/2016/8603964.

[40] .Aziz, O.; Shahcheraghi, F.; Salimizand, H.; Modrresi, F.; Shakibaie, M.R.;Mansour, S.; Ramazanzadeh, R.;Badmasti, F.;Nikbin, V. (2016). Molecular Analasis and Expression of bap Gene in Biofilm-Forming MultiDrug-Resistant Acinetobacter baumannii.Vol.5,1.

[41] .Al-Abdali, B. F. S. (2022). Studying the effect of gold nanoparticles on • some bacteria isolated from surgical wound infections. M.Sc. thesis, .University of Anbar. 167.

[42] .Kadhim, N. J., Al-Janabi, H. S., & Kadhim, M. J. (2020). Molecular 59- detection of Virulence Factors

genes Associated with Immune Resistance in Klebsiella pneumonia. Medico Legal Update, 20(3), 1459-1465.

[43] .Hussein, A. S. K. (2016) A bacteriological and molecular study of the species Proteus mirabilis isolated from clinical and animal sources, M.Sc. thesis, College of Education for Pure Sciences/Ibn al-Haytham - University of Baghdad.

[44] .Al-Zanga, I. A. A. (2012), A bacteriological and genetic study of Klebsilla spp. isolated from various diseases, M.Sc. thesis / College of Education for Pure Sciences - University of Diyala.

[45] .Al-Abdali I. H. A. (2019) The effect of olive fruit extracts, Ficus carica L., and Olea euroupaea on some types of clinically isolated pathogenic bacteria, M.Sc. thesis, College of Education for Girls, University of Anbar.

[46] .Al-Gharawi R. S. M. (2019) Effect of Cinnamomu zeylanicum Apium graveolens L. extracts and celery seeds on antibiotic-resistant bacteria isolated from urinary tract infections in females. M.Sc. thesis, College of Science, Al- University of Mustansiriya.

[47] .Sharma S and Kaur M. 2010. Antimicrobial activities of rhizobacterial strains of Pseudomonas and Bacillus strains isolated from rhizosphere soil of carnation (Dianthus caryophyllus cv. Sunrise). Indian J Microbiol., 50:229-232.

الفعالية التثبيطية لبعض المستخلصات البكتيرية ضد أنواع معينة من البكتيريا المقاومة للمضادات الحيوية والمنتجة للأغشية الحيوية والمعزولة من التهابات مختلفة في البيئة المحلية

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الخلاصة:

تضمنت الدراسة تقييم الفعالية التثبيطية لمستخلصات المذيبات العضوية (الايثانول ،الكلوروفروم، استات الاثيل) للمستقلبات الثانوية المستخرجة من البكتيريا (*Bacillus subtillus*، *Kytococcuc sedentarius*، *Pseudomonas Fluorescens*) المعزولة من البيئة المحلية في مدينة الرمادي ضد نمو البكتيريا . تم استخدام طريقة الانتشار للتحقق من المقاومة العزلات لعشرة مضادات حيوية ومنتجة الأغشية الحيوية. وقد تم عزلها من العينات السريرية (التهابات المسالك البولية، الإسهال، التهابات الجروح والحروق). أظهرت المستخلصات الكحولية فعالية مضادة للبكتيريا ضد مجموعة واسعة من البكتيريا، بما في ذلك البكتيريا إيجابية وغرام سلبية. أظهرت النتائج أن المستخلص الإيثانولي لبكتريا *Ps.Fluorescens* كان له تأثير كبير في تثبيط نمو بكتريا *Staphlococcus.aureuse*، حيث بلغ قطر منطقة التثبيط 2±23 ملم، وكان أقل فعالية من قبل بكتريا Ps.Fluorescens ضد بكتريا *Escherichia.coli* حيث كان قطر التثبيط 2±10 ملم. كما أظهرت النتائج أن مستخلص الكلوروفورم من بكتريا *K.sedentarius* كان له أعلى نشاط ضد بكتريا *Acintobacter baumanii* حيث بلغ 2±28 ملم. أظهرت النتائج أن المستخلص خلات الإيثيل لبكتريا *Bacillus subtillus* كان له تأثير كبير في تثبيط نمو بكتريا *S.aureuse*، حيث بلغ قطر منطقة التثبيط 2±31 ملم، وأقل فعالية ضد البكتيريا *klebsella pneumonia* ، حيث أنتجت قطر تثبيطي 1±20 ملم. من ناحية أخرى، كان لمستخلص خلات الإيثيل *Ps. Fluorescens* أعلى تثبيط ضد بكتريا *S.aureuse* مسجلاً 2±31 ملم، وأقل فعالية ضد بكتريا *pseudomonas.areuginosa* بلغت 1±9 ملم.

الكلمات المفتاحية: المستخلص البكتيري، التمثيل الغذائي الثانوي، البكتيريا المقاومة للمضادات الحياتية.