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Anti- Biofilm efficacy of *Agaricus bisporus* extract against clinical isolates of *Pseudomonas aeruginosa*

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Abstract

This study evaluated the anti-biofilm and antibacterial efficacy of *Agaricus bisporus* extract against ten clinical isolates of *Pseudomonas aeruginosa*. Antibiotic susceptibility testing via disk diffusion revealed 100% resistance to Gentamicin and 80% sensitivity to Levofloxacin, while the Congo Red Agar method confirmed that 30% of the isolates were biofilm producers. The *Agaricus bisporus* extract demonstrated variable, concentration-dependent antibacterial activity using the agar well diffusion method; at 100% concentration, it produced inhibition zones of 11-14 mm in four out of five tested isolates, whereas the 50% concentration showed inconsistent, moderate activity in only two isolates, and the 25% concentration exhibited weak or no activity across most plates.

Keywords

Pseudomonas Aeruginosa;
Biofilm;
Agaricus Bisporus;
Antibiotic Susceptibility;
Congo Red Agar



INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic, facultative anaerobe pathogen that has become an important cause of infection in humans and can be associated with significant morbidity and mortality. Severe infections usually affect immunocompromised patients or those with chronic debilitating diseases. Its significance as a pathogen is exacerbated by its resistance to antibiotics, virulence factors and its ability to adapt to a wide range of environments. *P. aeruginosa* can have multiple intrinsic or acquired mechanisms of resistance, frequently with high resistance rates to various antimicrobial classes.

In recent years, the worldwide spread of the so-called 'high-risk clones' of multidrug-resistant or extensively drug-resistant *P. aeruginosa* has become a public health threat that needs to be studied and managed with urgency and determination. Among Gram-negative infections, *P. aeruginosa* is one of the most common Gram-negative bacteria causing nosocomial and healthcare-associated infections in hospitalised patients. According to WHO guidelines, measures to prevent the transmission of multiresistant *P. aeruginosa* in healthcare facilities should include at least hand hygiene (with the appropriate use of alcohol-based solutions), contact precautions, patient isolation (single room or cohort), environmental cleanliness and surveillance. (Sartini, and Cristina, 2021).

The *Pseudomonas aeruginosa* genome (G + C content 65-67%, size 5.5-7 Mbp) is made up of a single circular chromosome and a variable number of plasmids. Sequencing of complete genomes or blocks of the accessory genome has revealed that the genome encodes a large repertoire of transporters, transcriptional regulators, and two-component regulatory systems which reflects its metabolic diversity to utilize a broad range of nutrients. (Klockgether, J. et al., 2011)

Identification of *P. aeruginosa* can be complicated by the fact individual isolates often lack motility. The

colony morphology itself also displays several varieties. The main two types are large, smooth, with a flat edge and elevated center and small, rough, and convex. A third type, mucoid, can also be found. The large colony can typically be found in clinical settings while the small is found in nature. The third, however, is present in biological settings and has been found in respiratory and in the urinary tract (Boone, D. R., 2001).

Agaricus bisporus belongs to Basidiomycetes family and the most important commercially cultivated mushroom in the world. The rich nutrients like carbohydrates, proteins, lipids, fibers, minerals, and vitamins present in this mushroom as famous healthy food. Moreover, because of the presence of some active ingredients, such as polysaccharides, lipopolysaccharides, essential amino acids, peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids and their derivatives, these mushrooms have been reported to have antimicrobial, anticancer, antidiabetic, antihypercholesterolemic, antihypertensive, hepatoprotective and antioxidant activities.

This study is focused on reviewing the recent studies published in the medical and nutritional properties of *Agaricus bisporus*. (Atila, F., & Shariati, M., 2017).

1.1 Nomenclature of *Pseudomonas aeruginosa*

The name *Pseudomonas aeruginosa* derives from the Greek *pseudēs* (false) and Latin *monas* (single unit), while *aeruginosa* is a Latin adjective meaning verdigris (copper rust). (Palleroni NJ, 2010).

1.2 Morphology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a heterotrophic, motile, Gram-negative rod-shaped bacterium (1-5 μm $\times$ 0.5-1.0 μm). It is a facultative aerobe that grows optimally at 37°C (range: 4-42°C), capable of utilizing over 100 organic compounds as carbon or energy sources. It is commonly found in soil, water, and hospital environments. (Diggle, S. P., & Whiteley, M., 2020).

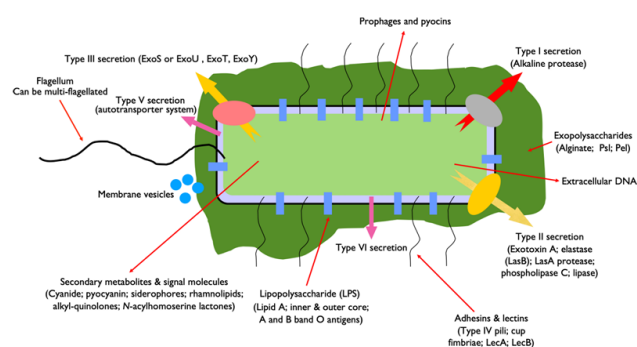


Fig 1-1 Morphology and component of *Pseudomonas aeruginosa*

1.3 Epidemiology of *Pseudomonas aeruginosa*

1.3.1. Cystic fibrosis

By adulthood, approximately 80% of cystic fibrosis (CF) patients become chronically infected with *Pseudomonas aeruginosa*. Over time, bacteria evolve phenotypically, acquiring increased antibiotic resistance. (Mahenthiralingam & Speert, 1996).

1.3.2 urinary tract infection

Pseudomonas aeruginosa accounts for 5–15% of UTIs in hospitalized patients, particularly those with indwelling catheters. Multidrug-resistant strains are increasingly reported worldwide. (Strateva T, Yordanov D, 2009).

1.3.3 Burn Wound Infections and Sepsis

Pseudomonas aeruginosa is the most common Gram-negative pathogen in burn wound patients, and a leading cause of sepsis and death. MDR strains accounted for 64% of sepsis deaths in pediatric burn ICUs. (Norbury W, 2016).

1.3.4 Bloodstream infections

Bloodstream infections due to *Pseudomonas aeruginosa* carry mortality rates of 43–59%, with MDR strains complicating 17–28% of cases. It is more commonly

hospital-acquired compared to other Gram-negative bloodstream infections. (Kang CI, 2005).

1.3.5 Surgical Site Infections

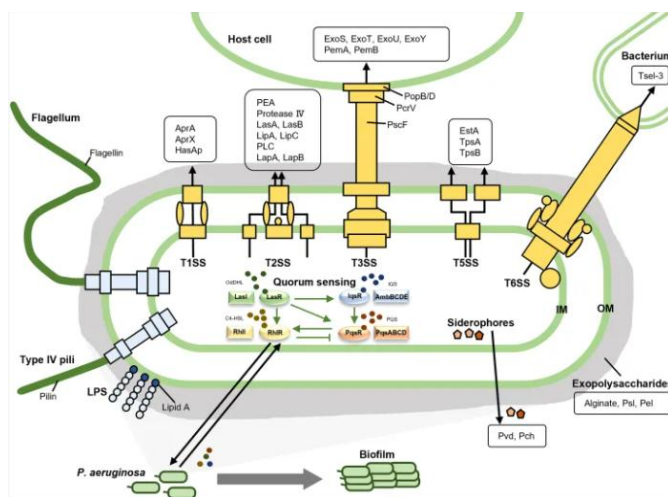
Pseudomonas aeruginosa accounts for approximately 5.7% of surgical site infections, particularly in breast and cardiac surgeries, and is associated with increased postoperative mortality. (Weiner LM, 2016).

1.3.6 Otitis

Pseudomonas aeruginosa is the primary cause of bacterial otitis externa. Strains from this infection resemble environmental types rather than those adapted to other human clinical infections. (Van Asperen et al., 1995).

1.4 Virulence Factors

The virulence factors of *Pseudomonas aeruginosa* include biofilm formation, quorum sensing, and the production of toxins and enzymes that facilitate host invasion, immune evasion, and antibiotic tolerance. (Liao, C. et al., 2022).

Fig1-2 Virulence Factors of *Pseudomonas aeruginosa*

1.5 *Pseudomonas aeruginosa* and antibiotics

Pseudomonas aeruginosa is intrinsically resistant to many antibiotics due to low cell wall permeability, and can acquire further resistance via efflux pumps, beta-lactamases (ESBLs and MBLs), target modifications, and mobile genetic elements. MDR strains, especially in cystic fibrosis, represent a major clinical challenge. (Lambert, P., 2002).

1.6 *Pseudomonas aeruginosa* Biofilm

Pseudomonas aeruginosa forms biofilms composed of microbial cells embedded in extracellular polysaccharides (EPS), protecting bacteria from antibiotics and host immune responses. This makes chronic infections particularly difficult to eradicate. (Flemming H-C et al., 2000).

1.7 Treatment Strategies for *Pseudomonas aeruginosa*

Treatment of *Pseudomonas aeruginosa* infections relies on antipseudomonal beta-lactams, aminoglycosides, and fluoroquinolones, often in combination. For MDR strains, colistin and fosfomycin serve as salvage therapies. Emerging alternatives include bacteriophage therapy, antimicrobial peptides, and nanoparticle-based drug delivery. (Driscoll JA et al., 2007).

1.8 Chemical composition of *Agaricus bisporus*

1.8.1 Protein and Amino acid

Agaricus bisporus contains approximately 29.14% protein, rich in alanine, glutamic acid, aspartic acid,

leucine, lysine, and other essential amino acids. (Muslat et al., 2014).

1.8.2 Carbohydrate and Fiber

Mannitol is the predominant carbohydrate in *A. bisporus*. Dietary fiber components, including chitin, hemicelluloses, mannans, and beta-glucans, contribute to its health-promoting properties. (Cheung, 2009).

1.8.3 Mineral content

Agaricus bisporus is a rich source of minerals including K, Fe, Zn, Cu, Na, Se, and Ca, with potassium and phosphorus as the main constituents. (Falandysz & Borovička, 2013).

1.8.4 Vitamins

Niacin is the most abundant vitamin in *Agaricus bisporus*, followed by riboflavin; B1, B3, and ascorbic acid are also present. Vitamin D is absent in mushrooms cultivated in dark conditions. (Bernas & Jaworska, 2016).

1.8.5 Fatty acids

Agaricus bisporus is low in fat but contains essential fatty acids, primarily linoleic, palmitic, and stearic acids. (Barros et al., 2008).

1.9 Medicinal importance of *Agaricus bisporus*

1.9.1 Anticancer

Extracts of *Agaricus bisporus* exhibit immunomodulating and anticancer properties, including inhibition of leukemia cell proliferation via apoptosis induction, and stimulation of interferon-

gamma (IFN- γ) in human peripheral blood mononuclear cells. (Jagadish et al., 2009).

1.9.2 Antidiabetic

Rich in dietary fibers and antioxidants (vitamins C, D, B12, polyphenols), *Agaricus bisporus* has demonstrated anti-inflammatory, antioxidant, and antidiabetic effects, reducing severity of

streptozotocin-induced diabetes in animal models. (Yamaç et al., 2010).

1.9.3 Antimicrobial

Methanol extracts of *Agaricus bisporus* exhibit antimicrobial activity against bacteria, yeasts, and dermatophytes. Aqueous protein extracts have shown significant antibacterial activity particularly against *S. aureus* and MRSA. (Tehrani et al., 2012).

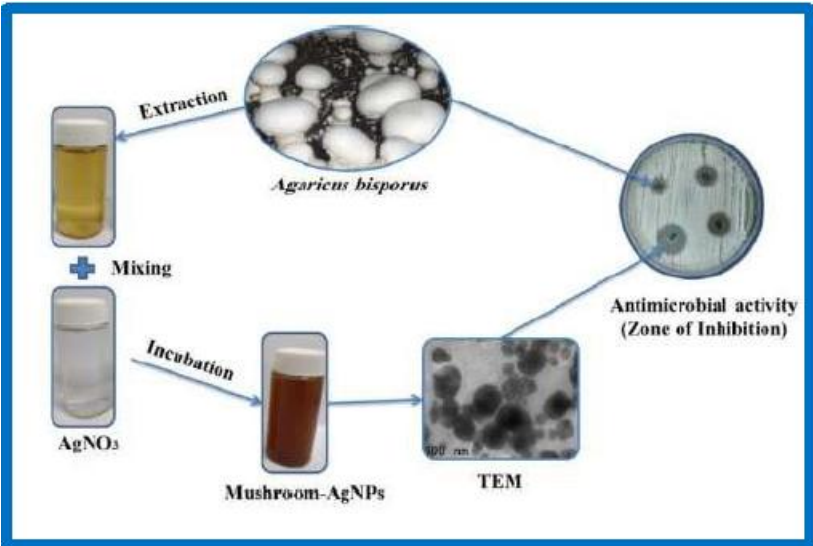


Figure 1-3 Biosynthesis silver nanoparticles using mushroom *Agaricus bisporus*

2. MATERIALS AND METHODS

2-1 Materials

2-1-1 Laboratory equipment

The apparatuses and the equipment used in this study were listed in table (2-1)

Table (2-1): The apparatuses and the equipment used in this study:

Apparatuses	Company	Origin
Autoclave	Hirayama	Japan
Deep Freeze	Binder	Germany
Hot plate	VELP	Germany
Incubator	Nuc	Denmark
Micropipettes	Eppendorf	Germany
Refrigerator	Kelon	Korea
Sensitive balance	Denver	European Union
distilled water	GFL	Germany
Soxhlet extractor	Scitek Global	Chain
microtiter plate	Eppendorf	Germany

fume hood	Waldner	Germany
Swabs	Jinan	Chain
Petri Dish	Jinan	Chain
Loop	Biologix Group Ltd	Chain

2-1-2 Chemicals, buffers and Biological Materials

All chemical and biological materials that used in this study were summarized in table (2-2).

Table (2-2): The chemical and biological materials used in this study

Chemical and materials	Company	Origin
Absolute ethanol 99.9%	Diamond®	France
Formalin	CDH	India
Catalase	Cavloerbar	France
Oxidase reagent (1%)	BDH	England

2-1-3 Stains and indicators

The stains and indicators used in this study were listed in (Table 2-3).

Table (2-3): The stains and indicators

Stains and indicators	Company	Origin
Congo red indicator	CDH	India

2-1-4 Antibiotics

The antibiotics disks used in the study were listed in table (2-4)

Table (2-4): The antibiotic disks

Antibiotic	Concentration	Symbol	Company	Origin
Piperacillin	100µg	PRL	Bioanalyse	Turkey
Tobramycin	10 µg	TOB	Masr group	United Kingdom
Levofloxacin	5 µg	LE	Bioanalyse	Turkey
Gentamicin	10µg	GEN	Bioanalyse	Turkey
Cefepime	30 µg	CPM	Bioanalyse	Turkey
Imipenem	10 µg	Ipm	Bioanalyse	Turkey
Aztreonam	30 µg	AT	Bioanalyse	Turkey
Ceftazidime	30 µg	CAZ	Bioanalyse	Turkey

2-1-5 Culture media

All culture media used for isolation and identification of bacteria in this study were listed in table (2-5).

Table (2-5): The culture media

Culture media	Company	Origin
Nutrient agar	Oxoid	U.K.
Nutrient broth	Mast group	U.K.
Mueller Hinton agar	Oxoid	England
Cetrimide	Mast group	U.K.

2-2 methods

2-2-1 Isolation and Culture of *Pseudomonas aeruginosa*

clinical samples were collected from patients suffering from UTI and Cystic fibrosis. The samples were subjected to microbiological analysis to isolate and identify *Pseudomonas aeruginosa*. Identification was based on cultural, morphological, and biochemical characteristics. Cetrimide Agar medium is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from, sputum and urine Specimen. in the process of primary isolation of the bacterium. These media are considered selective mediums for *P. aeruginosa* isolation it allowed the growth of *P.aeruginosa* .the *P.aeruginosa* grows as pigmented colonies (blue-green to yellow-green)

2-2-2 Preparation of culture media

All culture media that mentioned in table (2-5) were prepared according to the manufacturer's instructions, autoclaved at 121°C/pounds/inch² for 15 minutes, and then transferred into sterile test tubes or Petri dishes as needed. They were then sterilized for 24 hours at 37°C before being stored at 4°C until needed.

2-2-3 Laboratory-prepared culture media

2-2-3-1 Cetrimide Agar

Cetrimide Agar is a solid selective medium used for the isolation and identification of *Pseudomonas aeruginosa* from different material, modified. Cetrimide inhibits bacterial growth except *Pseudomonas aeruginosa* and enhances fluorescein and pyocyanin pigment production.

2-2-2-2 Nutrient agar

is a general-purpose solid culture medium commonly used for the cultivation and maintenance of non-fastidious bacteria. In this research, nutrient agar was used for the primary cultivation and sub-culturing of the clinical isolates of *Pseudomonas aeruginosa* in order to obtain pure and well-isolated colonies prior to experimental procedures. The medium supports adequate bacterial growth and allows the observation of colony morphology,

2-2-3-3 Mueller-Hinton agar

is a standardized microbiological medium widely used for antimicrobial susceptibility testing because it allows optimal diffusion of antimicrobial agents through the agar matrix.

2-2-3-4 Nutrient broth

Nutrient broth was chosen as the culture medium because it provides a rich supply of nutrients, allowing the bacteria to grow uniformly in suspension. Unlike solid media, broth cultures facilitate better bacterial dispersion and ensure an adequate bacterial load for subsequent experiments.

2-2-4 Reagents

2-2-4-1 Oxidase reagent

It consists of N, N, N, N-Tetramethyl paraphenyldiamins dihydrochloride and prepared by dissolving (1) g of the substance in (100) ml of distilled water.

2-2-4-1 Catalase reagent

Prepared hydrogen peroxide solution (H₂O₂) (3%).

2-2-5 Testing for antimicrobial susceptibility

antimicrobial susceptibility testing using disk diffusion (Kirby-Bauer) was performed to detect the sensitivity of the isolates. *P. aeruginosa* isolates were inoculated into the Mueller-Hinton agar culture medium at a turbidity of 0.5 McFarland standards, and antibiotic disks were placed on the agar surface. After 24 hours of incubation at 37°C. The results were classified as sensitive (S), intermediate (I), or resistant (R) based on the zone of bacterial inhibition measured in millimeters around the disk. The results were compared with the reference values for antibiotic inhibition zones, as viewed in Table (2-6). The disks used in the antimicrobial susceptibility test mentioned in table (2-4)

1) Preparation of Inoculum

The inoculum was prepared by transferring colonies from an overnight culture of *P. aeruginosa* isolates to a 5ml tube of Nutrient broth and adjusting to a 0.5 McFarland Tube to obtain a culture with 1×10^8 cfu/ml.

2) Test plates inoculation

1-Plates were swabbed by dunking a sterile swab into the inoculum, prior to inoculation, remove excess broth from the swab by pressing and rotating the swab firmly against the side of the tube above the fluid level.

2 -The swab was rubbed over the medium's surface three times after each application by turning the plate through at a 60-degree angle. Eventually, the swab was wrapped around the edge of the agar surface.

3- The inoculum was left to dry at room temperature for a few minutes with the lid closed.

4 - Using sterile forceps, antibiotic discs were placed on the inoculated plate. The discs warmed to room temperature before being placed on the agar surface and gently pressed down with sterile forceps. The plates were incubated for 48 hrs. at 37°C

Table (2-6): The interpretation standards for zone diameter

Antimicrobial Agent	Disc Conc. µg/disk	Diameter of Inhibition Zone (mm)		
		Sensitive	Intermediate	Resistant
Piperacillin	100 µg	≥18	15-17	≤14
Tobramycin	10 µg	≥17	13-16	≤12
Gentamicin	10 µg	≥25	20-24	≤19
Cefepime	30 µg	≥20	15-19	≤14
Levofloxacin	5 µg	≥16	13-15	≤12
Imipenem	10 µg	≥23	20-22	≤19
Ceftazidime	30 µg	≥18	15-17	≤14
Aztreonam	30 µg	≥22	16-21	≤15

2-2-6 Assay for biofilm formation (qualitative method)

(CRA) method was used as a qualitative technique to detect the ability of bacteria to produce biofilm or extracellular polysaccharides. This method is

commonly applied for screening biofilm-forming bacteria such as *Pseudomonas aeruginosa*.

In this study, we prepared the medium by dissolving nutrient agar in distilled water and heating it until the components were completely dissolved. The medium was then sterilized by autoclaving. Meanwhile, the

Congo red dye solution was prepared separately in distilled water and sterilized. After the sterilized agar medium cooled to approximately 55 °C, we added the sterile Congo red solution and mixed it thoroughly to ensure uniform distribution of the dye. The prepared medium was then poured into sterile Petri dishes and allowed to solidify. A fresh bacterial isolate was streaked onto the surface of the Congo Red Agar plate using a sterile inoculating loop. The plates were incubated aerobically at 37 °C for 24-48 hours. After incubation, we examined the colonies carefully and recorded their color and colony morphology in order to determine the ability of the isolates to form biofilm.

2-2-7 Preparation of *Agaricus bisporus* extract

Agaricus bisporus was collected from local markets in Baghdad and subjected to a drying process for over

a week to remove moisture content. After complete drying, the fungal extract was obtained using the Soxhlet extraction viewed in (Figure2-1) extracts was done according to (R. Abubakar & M. Haque2020) In this process, full 20 g dry powder of each *Agaricus bisporus* prepared by using Soxhlet apparatus, was put in thimble. All extracts were kept in serial tube at 4C until use method to ensure efficient extraction of bioactive compounds as viewed in (Figure2-2) The crude extract was then prepared at different concentrations (100, 50, and 25 mg/mL) to evaluate its antimicrobial potential against *Pseudomonas aeruginosa*. we use Mueller-Hinton agar The bacteria were first inoculated into the medium. After that, four wells were carefully made in the center of the agar plate. One well was used as a control, while the remaining wells were filled with different concentrations of the *Agaricus bisporus* extract.

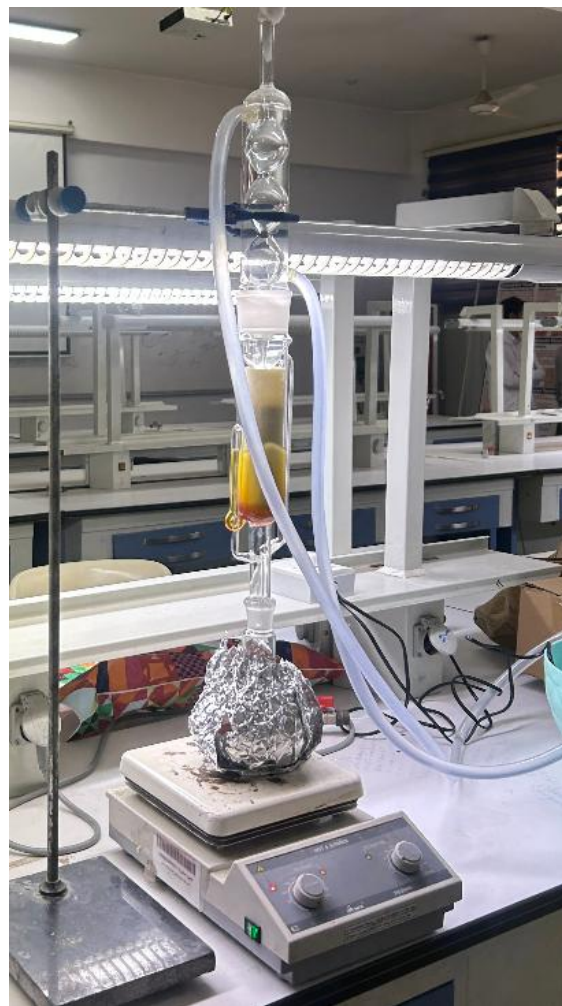


Fig 2-1 Soxhlet extraction



Fig 2-2 *Agaricus bisporus* extract

3. RESULTS AND DISCUSSION

3-1 Isolation and identification of *pseudomonas aeruginosa*

3-1-1 Culture of *pseudomonas aeruginosa*

Isolation medium Cetrimide Agar medium is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from, sputum and urine specimens. in the process of primary isolation of the bacterium. These media are considered selective mediums for *P. aeruginosa* isolation it allowed the growth of *P. aeruginosa* and suppresses the growth of other bacteria Where *P. aeruginosa* colonies may appear after an incubation period (24) hour at a temperature of 37 °C as pigmented blue, bluegreen or nonpigmented colonies as can be seen in (Figure 3-1).

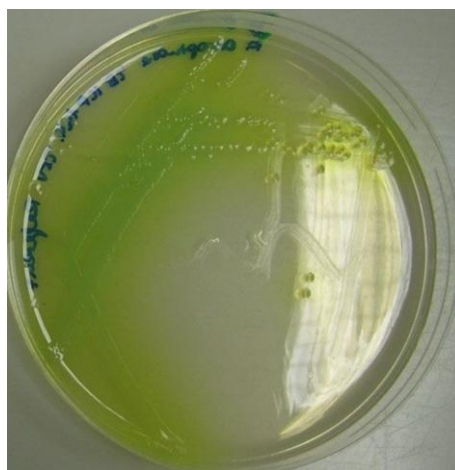


Fig 3-1 *Pseudomonas aeruginosa* in Cetrimide Agar

3-1-2Biochemical tests

The isolates were subjected to catalase and oxidase tests. All the positive culturable isolates gave positive results for catalase and oxidase. Most bacteria of the *P.aeruginosa* genus can produce catalase enzyme which can decompose H₂O₂ into water and oxygen, which appeared as bubbles As shown in (Figure 3-2)

Also, *P.aeruginosa* had the ability to produce an oxidase enzyme that assayed for the presence of cytochrome oxidase. When *P. aeruginosa* isolates contain the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product. as shown in (Figure 3-2)

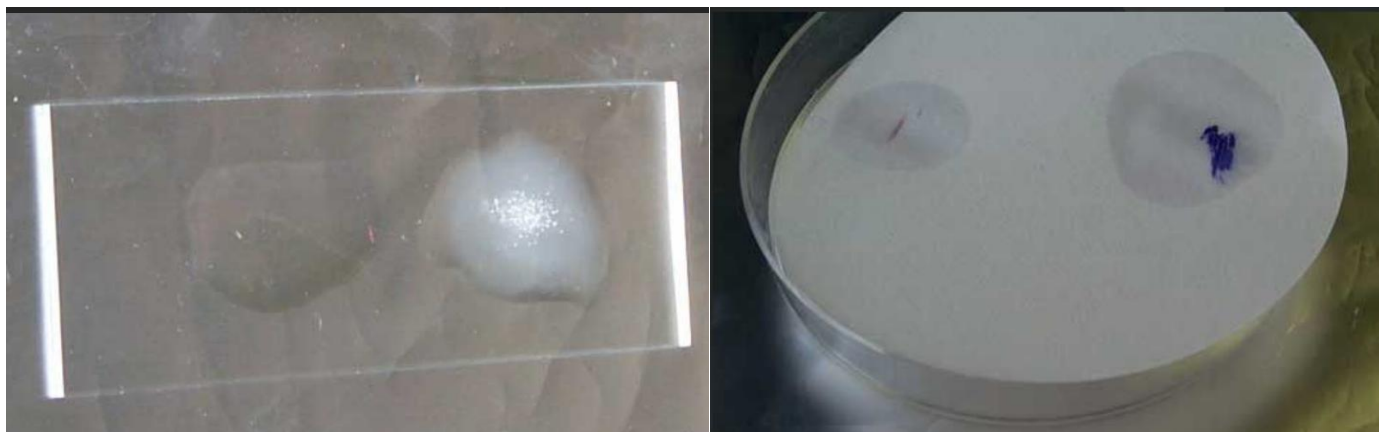


Fig 3-2 catalase and oxidase tests Result of *Pseudomonas aeruginosa*

3-2 the Result of antibiotic against *Pseudomonas aeruginosa*

Ten different *Pseudomonas aeruginosa* isolates were used in this study. Each isolate was cultured on Mueller-Hinton agar, after the antibiotic discs that list in table (2-4) was applied to the agar surface. The

plates were then incubated for 24 hours. After the incubation period, the antibacterial activity was evaluated by observing and measuring the zones of inhibition the viewed in (Fig 3-1), we measured using a regular ruler and the results were recorded as follows in table (3-1).

Table 3-1 the Result of antibiotic against *Pseudomonas aeruginosa*

	GEN	CAZ	LE	PIT	IPM	CPM	AT	TOB
1	15mm	13mm	18mm	25mm	20mm	25mm	25mm	
2	12mm	26mm	27mm	zero	25mm	31mm	28mm	20mm
3	12mm	25mm	26mm	30mm	28mm	32mm	37mm	22mm
4	14mm	24mm	27mm	32mm	27mm	29mm	27mm	30mm
5	10mm	33mm	29mm	35mm	29mm	26mm	33mm	23mm
6	zero	zero	zero	zero	Zero	zero	zero	zero
7	13mm	18mm	20mm	39mm	30mm	29mm	35mm	23mm
8	15mm	20mm	27mm	zero	23mm	zero	zero	zero
9	zero	zero	zero	zero	Zero	zero	zero	zero
10	12mm	14mm	24mm	26mm	20mm	22mm	22mm	27mm

antibiotic	Sensitive	Intermediate	Resistant
Gentamicin (GEN)	0/10 (0%)	0/10 (0%)	10/10 (100%)
Ceftazidime (CAZ)	6/10 (60%)	0/10 (0%)	4/10 (40%)
Levofloxacin (LE)	8/10 (80%)	0/10 (0%)	2/10 (20%)
Piperacillin (PIT)	6/10 (60%)	0/10 (0%)	4/10 (40%)
Imipenem (IPM)	7/10 (70%)	1/10 (10%)	2/10 (20%)
Cefepime (CPM)	7/10 (70%)	0/10 (0%)	3/10 (30%)
Aztreonam (AT)	7/10 (70%)	0/10 (0%)	3/10 (30%)
Tobramycin (TOB)	6/9 (66.7%)	0/9 (0%)	3/9 (33.3%)

Percentage of antibiotic effect on *Pseudomonas aeruginosa*

High resistance (>70%): Gentamicin (GEN) 100% (10/10 isolates) showed the highest resistance among the tested antibiotics.

Moderate resistance (40-60%): Ceftazidime (CAZ) 40% (4/10 isolates) and Piperacillin (PIT) 40% (4/10 isolates) and Tobramycin (TOB) 33.3% (3/9 isolates tested) showed moderate levels of resistance among the isolates.

Low resistance (<30%): Levofloxacin (LE) 20% (2/10 isolates), Cefepime (CPM) 30% (3/10 isolates), Aztreonam (AT) 30% (3/10 isolates), Imipenem (IPM) 20% (2/10 isolates),

Levofloxacin (LE) demonstrated the highest sensitivity among the tested antibiotics, with 80% (8/10 isolates) being sensitive, suggesting that it may be one of the most effective antibiotics against the tested *Pseudomonas aeruginosa* isolates.

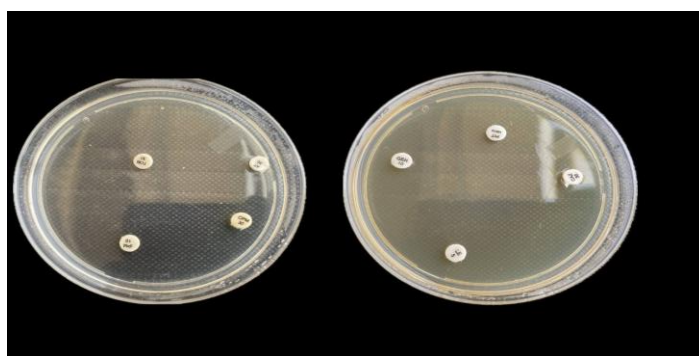
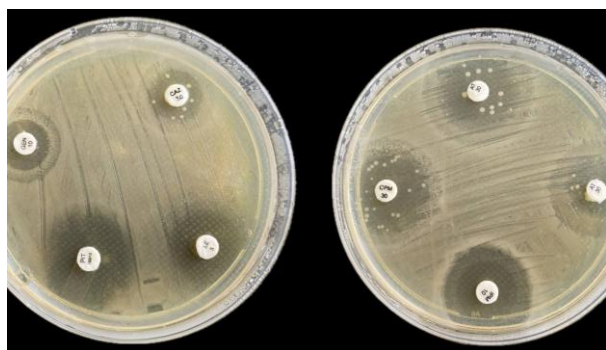


Fig (3-3): Inhibition zones of some *Pseudomonas aeruginosa* isolates against antibiotics

Based on these findings, and in comparison, with the results of the experiment Javiya (Antibiotic susceptibility patterns of *Pseudomonas aeruginosa*), several observations can be identified.

Aminoglycosides: Javiya reported high sensitivity for amikacin, whereas our study showed complete resistance to gentamicin (100%) and moderate resistance to tobramycin (33.3%), indicating variable efficacy within the same class.

Cephalosporins: Javiya emphasized improved sensitivity with β -lactamase inhibitors, while our study demonstrated moderate resistance to ceftazidime (40%) and lower resistance to cefepime (30%), reflecting differences between generations.

Penicillins: Javiya considered semi-synthetic penicillins effective when combined with inhibitors, but our study showed moderate resistance to piperacillin (40%) without mention of inhibitor use. (Javiya, V et al.,2008).

3-3 The results of Biofilm by *Pseudomonas aeruginosa*

Ten bacterial isolates were used. After 24 hours of bacterial inoculation on plates containing Congo Red stain and subsequent incubation for an additional 24 hours, three bacterial isolates produced visible

biofilm on the culture plates (30%), as shown in the figure (3-2).

These results are consistent with Lima study.in his study he using twenty isolates, which, when cultured on Congo Red agar, demonstrated a biofilm production rate of 15%. (Lima, J. et al.2017)

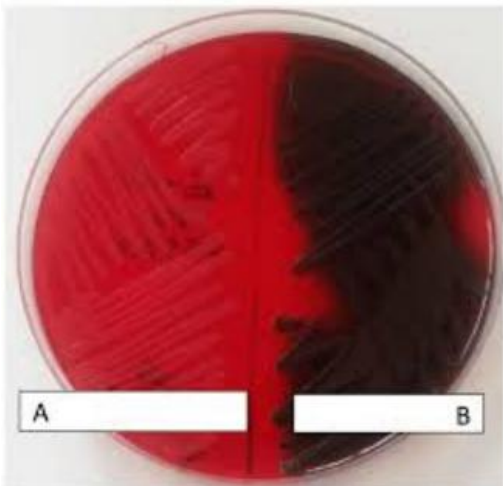


Fig (3-4): Biofilm production by *Pseudomonas* isolates on Congo Red Agar

3-4 the Result of *Agaricus bisporus* extract against *Pseudomonas aeruginosa*

The findings of this study demonstrate that the bioactive compounds present in *Agaricus* exhibit

significant antibacterial activity against *Pseudomonas aeruginosa*. After 24 hours of incubation. The results were as shown in the table. (3-3)

Table 3-3 Result of *Agaricus bisporus* extract against *Pseudomonas aeruginosa*

	25%	50%	100%
1 (1)	zero	zero	11mm
2 (2)	13mm	15mm	12mm
3 (95)	zero	zero	Zero
4(98)	9mm	18mm	12mm
5 (94)	zero	zero	14mm

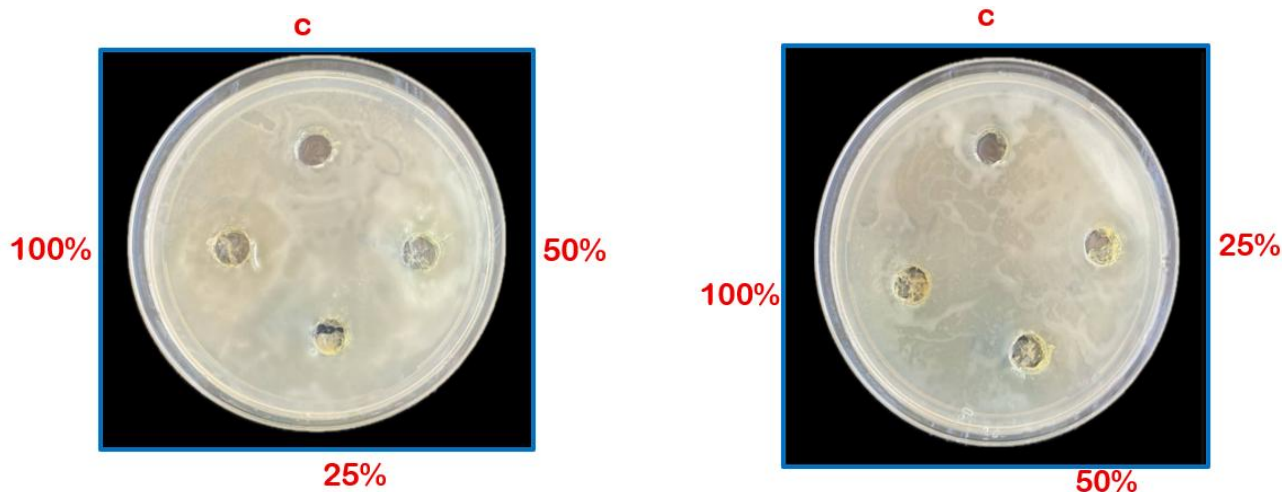


Fig (3-5): *Agaricus bisporus* extract against *Pseudomonas aeruginosa*

Clear inhibition zones were observed around bacterial cultures treated with *Agaricus* extract, indicating its ability to suppress *P. aeruginosa* growth. The size of the inhibition zones varied depending on the concentration of the extract, with the highest concentration (100%) showing the most pronounced antibacterial effects. However, at the 50% concentration, the antibacterial activity was inconsistent: while two isolates (plates 2 and 4) showed moderate inhibition (15 mm and 18

mm, respectively), the remaining three isolates (plates 1, 3, and 5) exhibited complete resistance (zero inhibition). Lower concentrations (25%) exhibited weak or no inhibition in most plates. These results highlight a dose-dependent response, though with notable variability among isolates, suggesting that the extract's efficacy is influenced by both the concentration of active compounds and the intrinsic susceptibility of the bacterial strain.

CONCLUSIONS

This study demonstrates that *Agaricus bisporus* extract possesses antibacterial activity against *P. aeruginosa* in a dose-dependent manner, with the highest efficacy observed at 100% concentration. However, the variability in response among isolates, particularly at lower concentrations, suggests that the extract's effectiveness may be influenced by the intrinsic susceptibility of the bacterial strain. Notably, three isolates were capable of biofilm formation, indicating their potential pathogenicity. These findings suggest that *Agaricus bisporus* extract could be a promising natural alternative for controlling *P. aeruginosa* infections, including biofilm-forming isolates, though further studies are needed to optimize its application and understand its mechanism of action.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

Further Purification and Characterization: Future studies should focus on the purification and chemical characterization of the bioactive compounds present in *Agaricus bisporus* extract responsible for the antibacterial activity against *Pseudomonas aeruginosa*. Identifying the specific active components could lead to the development of novel therapeutic agents.

Mechanism of Action Studies: In-depth investigations are needed to understand the exact mechanism by which *Agaricus bisporus* extract exerts its antibacterial and anti-biofilm effects. Elucidating whether the extract disrupts cell wall

synthesis, inhibits protein synthesis, or interferes with quorum sensing pathways would provide valuable insights.

Biofilm-Specific Assays: More specialized techniques such as the microtiter plate assay or confocal laser scanning microscopy should be employed to quantitatively assess the anti-biofilm activity of the extract and its ability to disrupt established biofilms, not just prevent formation.

Antibiotic Stewardship: The high level of gentamicin resistance (100%) observed in this study highlights the urgent need for antibiotic stewardship programs in healthcare settings to monitor and regulate antibiotic use, thereby slowing the emergence and spread of resistant strains.

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