

Antimicrobial activity of blue mold (*Penicillium italicum*) Filtrates against some species of pathogenic bacteria

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Abstract

Background: *Penicillium* saprophytic species, which primarily consume organic biodegradable materials, is a common example of a fungal species. The preparation filtrates of *Penicillium italicum*, a saprophyte on citrus fruits frequently linked to post-harvest diseases in this crop, were included in the current investigation.

Objective: This investigation aimed determine the most efficient culture medium for the production of antibacterial secondary metabolites.

Patients and Methods: included identifying the growth medium for *P. italicum* and produce its metabolites through the use of gas chromatography-mass spectrometry (GC MS) for both solid-state fermentation filtrate (SSFF) and liquid fermentation filtrate (LFF). Additionally, the antimicrobial activity of the mold filtrate against certain pathogenic bacteria was assessed using the agar well diffusion method.

Results: indicated that the biomass used for mold growth was heavier in SSFF than LFF, and according to the findings, the selective active isolate's crude filtrate from two duplicates of the *P. italicum* Yeast Extract Sucrose YES culture medium was 0.063 mg, while the crude extract from rotten orange (as a solid medium) containing *P. italicum* was 0.11 mg. Tetracosane and other substances with a track record of therapeutic activity were found in the two mold extracts, according to GC MS data. Overall, both SSFF and LFF demonstrated antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with the inhibition zones \pm standard deviation ($IZ \pm SD$) being 24.7 ± 0.57 , 18.2 ± 0.28 , 26.3 ± 0.59 , 21.6 ± 0.51 , and 32.8 ± 0.21 (for SSFF) mm and 0.0 , 12.3 ± 0.57 , 28.16 ± 0.20 , 19.3 ± 1.15 , and 28 ± 0.2 (for LFF) mm, respectively.

Conclusion: the filtrate of *P. italicum* from a natural medium (rotted orange) as a solid state fermentation was more weighted and gave many effective metabolites compared to what was produced by liquid fermentation on a synthetic medium, and both liquid and solid fermentation filtrates demonstrated efficacy against harmful bacteria.

Keywords: *Penicillium italicum*, SSFF, LFF, GC MS, antibacterial activity.

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Introduction

P. italicum is the primary citrus post-harvest phytopathogen that causes the blue mold infection. Citrus is one of the most significant fruit genera in the world. Due to unfavorable weather patterns and an increase in mold infections, orange production has significantly decreased recently (1,2). Because oranges are an acidic fruit (pH of 4-5 in healthy varieties), fungi are more likely than bacteria to cause rot in most oranges (3,4). However, mycotoxins a low molecular mass secondary metabolites generated by filamentous fungi that are poisonous to the host and other microorganisms sharing the same environment can be created and multiplied by phytopathogenic (plant-pathogenic) fungi (5). One of the most common types of fungi is penicillium, which grows on a variety of decomposing materials. Because Penicillium conidia are always present in the air, cultures frequently become contaminated by Penicillium colonies. Penicillin was discovered by coincidence; *P. italicum* and *P. digitatum* cause citrus fruits to rot, whereas *P. expansum* causes brown rot in apples (6,7). Among the most well-known examples of fungi are penicillium saprophytic species, which primarily feed on organic biodegradable materials. These species can grow on foods and other stored seeds because they prefer to flourish in low-humidity environments and to spread quickly through aerial dispersal when the seeds are appropriately moist (8). *P. italicum* is commonly associated with Citrus fruits and in the agricultural sector, It is an acute wound pathogen that affects all species and varieties of Citrus and can infect fruit in the field, packing house and even during distribution and marketing (9). Traditional fungal fermentation is being used today to produce foods and beverages all over the world. Penicillium species are utilized in Europe to help cheeses and meats mature (10). However, solid state fermentation has benefits over submerged (liquid) fermentation, including: greater volumetric productivity, typically simpler and requiring less energy; potential ease of meeting aeration requirements; resemblance to certain fungi's and

bacteria's natural habitat; simpler downstream processing; fungal hyphae are submerged in a liquid medium, preventing desiccation; Temperature control is usually not too difficult, allowing the organism to be exposed to a consistent temperature throughout its growth cycle; O₂ availability to the biomass can be reasonably well controlled at a specific level of medium saturation; nutrient availability to the organism can be controlled within relatively narrow limits if desired through the feeding of nutrient solutions; although shear forces do occur in mechanically stirred bioreactors, the nature and magnitude of these forces are well understood, and low-shear environments, like bubble columns or air lift bioreactors, can be used if the organism is highly susceptible to shear damage Lastly, it is not too difficult to give pH control (11, 12). The current study aims to identify the active medium for *P. italicum* metabolite growth and production using GC MS technology and to identify the mold filtrate's antibacterial activity against a few harmful bacteria. According to Webster and Weber classification (6). Penicillium italicum belongs to Kingdom fungi, phylum Ascomycota, class Eurotiomycetes, order Eurotiales and family Trichocomaceae.

Patients and Methods

The laboratory methods were performed in the Lab. of Fungi and Natural products at the department of Biotechnology/college of Sciences / University of Diyala.

Sampling and Identification of *Penicillium italicum*

From naturally rotting citrus fruits, fifty mold isolates were isolated. Citrus sinensis L.) In Baqubah city, Diyala province, Iraq), all isolates were cultured on Sabouraud Dextrose

Agar SDA for primary isolation, then sub-cultured on Czapic Dox Agar CZA for identification. Lactophenol Alanine Blue stain was used in the laboratory to identify the fungi based on the following criteria: Colony morphology, which includes color and consistency, reverse color, which changes with age, and microscopic features, such as conidial size, arrangement, form, and ontogeny. The selected isolate of *P. italicum* was subcultured and preserved in Potato Dextrose Agar slants and then placed as stock cultures at 4°C (13).

Growth conditions for *Penicillium italicum* and metabolite identification

➤ **Preparation spore suspension of *P. italicum***

After growing the mold for seven days at 28 ± 0.5 °C, the spores were harvested by stirring the culture with a sterile 0.85% NaCl, the conidia suspension was gently probed with a pipette tip and filtered to separate conidia from hyphal fragments.

➤ **Preparation of *P. italicum* filtrate by solid state fermentation**

A method was followed by Richard and Mary (14) according to the following steps with slight modifications:

- Twenty-five grams of each orange fruit *Citrus sinensis* L. (which were collected from a local market of Baqubah city and exposed to the ambient air for contamination 25 °C, 56 % relative humidity) in 250 ml cotton-stoppered Erlenmeyer flask which was sterilized by sodium hypochlorite and used for *Penicillium* filtrate production as solid substrate. The moisturizing ratio is 5:1 (w/v) by D.W.
- The selected isolate spores were fermented by growing on un-infected samples of chopped *C. sinensis* L. fruit (which was inoculated with 1 ml of spore suspension and put in an Erlenmeyer flask then incubated in state cooling incubator at 28 ± 2 ° C for two months (January and February).
- Fruit that had fermented with homogenizing using an electric magnetic stirrer for 10 minutes was given

75 milliliters of chloroform to complete the extraction process from rotten oranges (solid fermentation). Next, the extracted solution was filtered using a Whatman filter paper No. 1 and 50 ml of chloroform through a separating funnel. Following that, the filtrate fractions were combined and dried at 45°C by evaporation. The dried filtrate was kept in storage at 4°C (15).

➤ **Preparation of *P. italicum* filtrate by liquid fermentation**

- The selected isolate spore was secondly fermented by growing on the liquid culture of YES (prepared by dissolving 40 g of Sucrose and 20 g of yeast extract in 1000 ml of distilled water and sterilized by autoclave). The mixture was then allowed to incubate for ten days at 28 ± 2 ° C in a state cooling incubator. One milliliter of spore suspension was added to the medium to create two replicates for culture.
- 100 milliliters of liquid culture and 50 milliliters of chloroform were added to an Erlenmeyer flask, which was then electro-homogenized for ten minutes with the use of a magnetic stirrer. A Whatman filter paper No. 1 was then used to filter the extracted solution, and a separating funnel was used to filter 50 ml of chloroform. The separated components were combined and dried at 45°C using evaporation. Dried filtrate was stored at 4°C (8,15).

Evaluation of *Penicillium italicum* filtrates quantitatively

A sensitive electric balance was used to weigh the filtrates after they had been collected and dried to compare the different types.

Determination of the qualitative assessment of *Penicillium italicum* filtrates by Gas chromatography – Mass Spectrum analysis

Equal volumes of the two dried filtrates of the mold prepared in the previous paragraphs were

weighed to compare the types of filtrates. Then, to make the fungal filtrate stock solution for chemical analysis, each dried mold filtrate was dissolved in chloroform. GC–MS analysis of *Penicillium italicum* filtrates was performed on a GC system (Agilent 7890A series, USA). Helium (He), the carrier gas, was allowed to flow at a rate of 1 mL min⁻¹, with a split ratio of 1:50. While the detector temperature was fixed at 280°C, the injector temperature was altered to 250°C. The National Institute of Standards and Technology (NIST, USA) database was used to interpret the mass spectrum.

Determination of the antibacterial activity of *Penicillium italicum* filtrates

➤ Preparation of bacterial isolates

Pathogenic bacterial isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* of multidrug-resistant to antibiotics were obtained from Teaching Laboratories at Baqubah Teaching Hospital in Diyala province.

➤ Measurement of antibacterial activity

The agar well diffusion method was used according to Obeidat et al (16) with slight modifications as follows:

- Bacterial suspension for each species was prepared by transporting several bacterial colonies with a loop and put in a test tube containing brain heart infusion broth for activating the bacteria, the tube was then incubated at 37°C 18-24 hrs (17).
- The bacterial suspension was compared to the standard McFarland solution which is equal to 1.5 x 10⁸ CFU ml⁻¹. After that, the suspension of bacteria was spread by sterile swab on the plates containing Muller Hinton Agar and the plate was then left to be dried.
- Three 5mm diameter holes were made in the culture medium using a sterilized cork borer.
- A concentration of 50 mg ml⁻¹ of each fungal

filtrate was made using DMSO 10% by dissolving 250 mg of dry filtrate in 5 ml of DMSO 10%, to obtain a concentration of 50 mg ml⁻¹.

- 100 µl of the concentration of the test filtrates were added to the holes individually by micropipette. The third pit (control) was represented by adding DMSO 10%, and three replicates worked each dish. After that, incubate the dishes at 35±2 °C for 18-24 hrs.
- The effectiveness of each filtrate was determined by measuring the inhibition zone (IZ) diameter around each hole and then compared with the control.

Statistical analysis

Analysis of variance (ANOVA) using statistical software. P < 0.001, 0.02, 0.05 and 0.06 values were used for the statistical tests as a significance level.

Results

Twenty-two of the fifty mold isolates that obtained through culturing on SDA were recognized as *Penicillium italicum* and twenty-eight as *P. digitatum* from naturally rotting citrus fruits. Microscopic examination showed that the conidial apparatus is made up of asymmetric penicilli that bear tangled chains of conidia; conidiophores, which are more or less cylindrical, smooth-walled, terverticillate metulae that bear three to six phialides each, originate from the substratum or occasionally from superficial hyphae. The phialides have cylindrical, short necks that are easily distinguished. Conidia are 4.0–5.0 × 2.5–3.5 µm in size, smooth, greenish, and have smooth walls. Phialides are generated single, in groups, or from branched metulae, giving them a brush-like look (a penicillus). Figure 1 shows the microscopic and macroscopic characteristics of this mold.

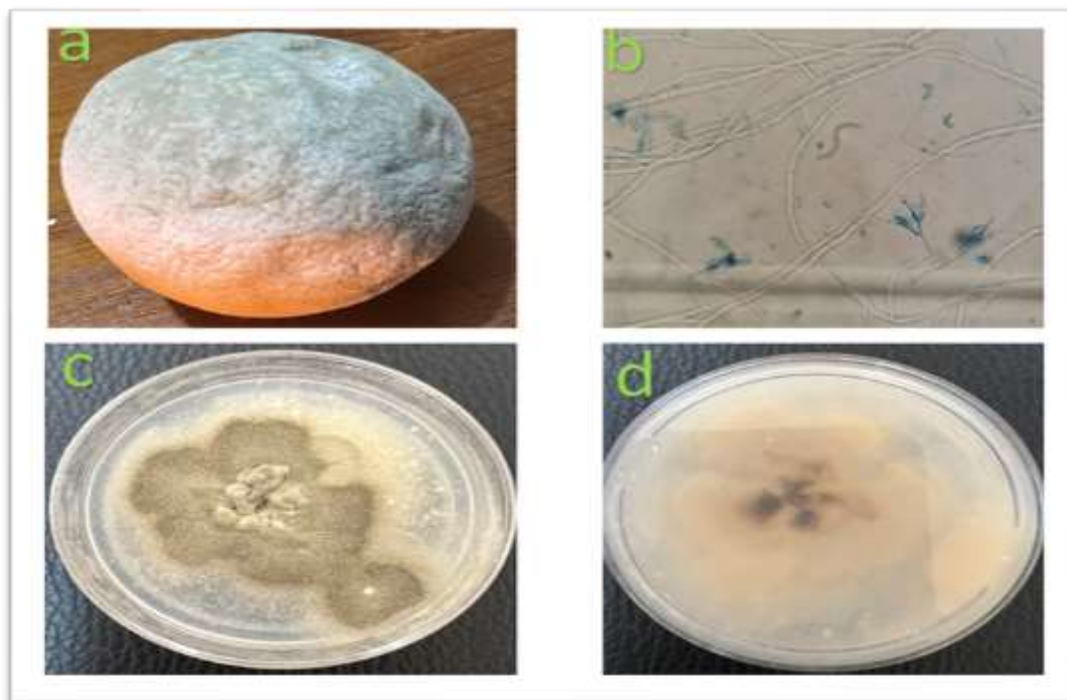


Figure 1:

Penicillium italicum a Rotten orange *Citrus sinensis* L. with the mold. b, microscopic view of the mold stained with Lactophenol cotton blue 40X. c, macroscopic top appearance grew on CZA at $28\pm 2^{\circ}\text{C}$ and pH 5.6 for 7 days of incubation, d-reversed view.

For quantitative estimation of growing *Penicillium italicum* filtrates metabolites, 0.11 mg of *P. italicum* was present in the rotten orange crude extract (filtrate). On

the other hand, the chosen isolate of this mold contained 0.063 mg of crude extract from two replicates of *P. italicum* YES growth media. Figures 2 and 3 depict the process of fermentation and filtrate extraction.

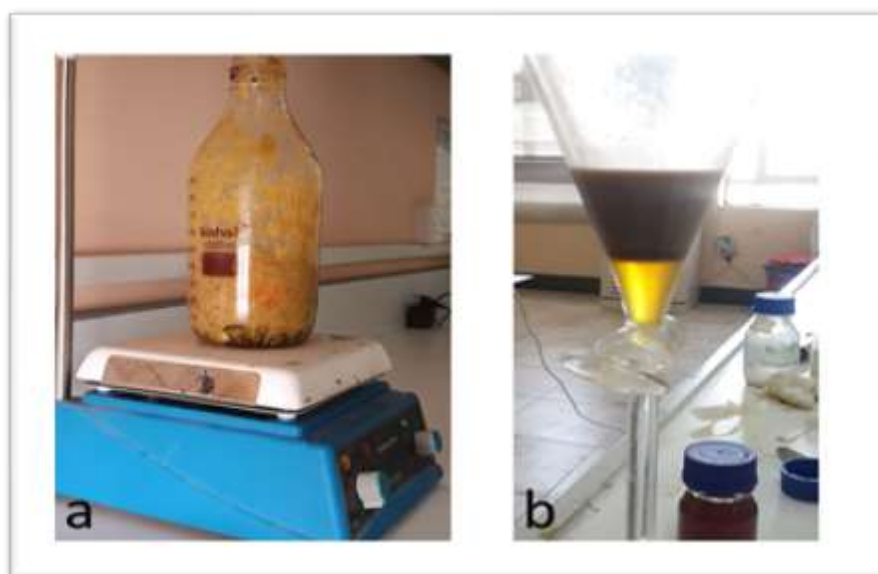


Figure 2: a, homogenizing *Penicillium italicum* on the solid-state fermented orange by magnetic stirrer b, extraction of *P. italicum* filtrate by separating funnel using Chloroform

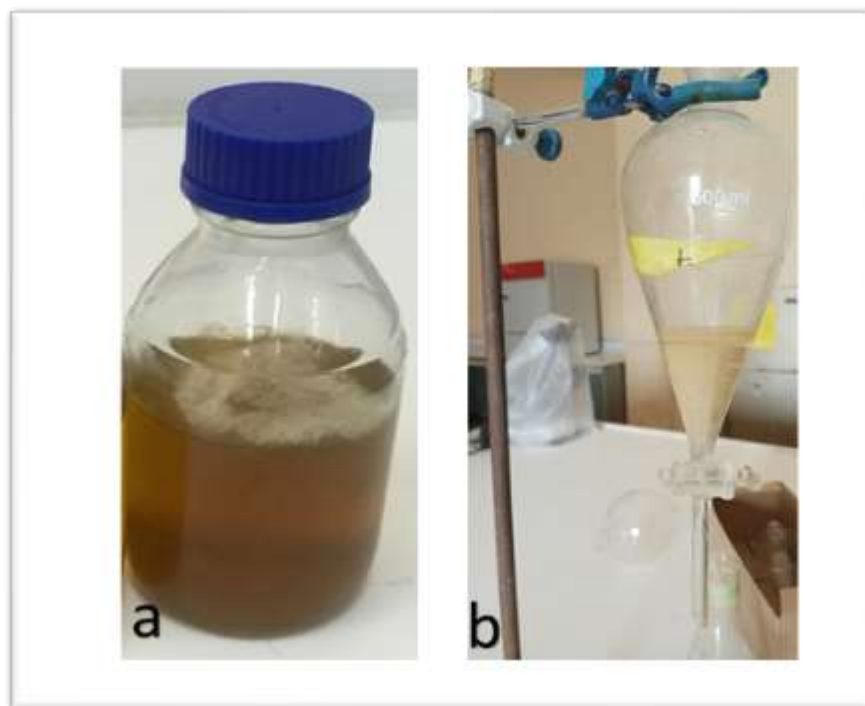


Figure 3: Liquid fermented *Penicillium italicum* YES medium b, extraction of *P. italicum* filtrate by separating funnel using Chloroform

Through quantitation chemical analysis of *P. italicum* chloroformic extract; results showed that sixteen compounds were identified in the liquid fermentation filtrate LFF and thirty-one compounds

were detected in the solid-state fermentation filtrate SSFF (tables 1 and 2). The identification of chemical compounds was based on the peak area, retention time, molecular weight and chemical structure.

Table 1: GC MS analysis of *Penicillium italicum* liquid fermentation filtrate for chemical compounds detection

No	RT (min)	Area%	Name	Quality	CAS Number	M.W
1	23.933	3.67	2-HYDROXY-3,5,5-TRIMETHYL-2-CYCLOHEXENONE	50	004883-60-7	154.21
2	25.147	9.27	2-(n-Butyl-N-(2-methylpropanoyl)amino-4-methyl-oxazole	37	000000-00-0	1422.7
3	25.428	30.28	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	59	004883-60-7	154.21
4	27.923	7.15	methyl dihydromalvalate	46	000000-00-0	294.5
5	30.741	2.67	Iron, tricarbonyl[N-(phenyl-2-pyridinylmethylene)benzenamine-N,N']-	90	074764-11-7	398.2
6	32.1	4.28	Tetracosane	98	000646-31-1	338.65
7	32.764	4.23	Benzonitrile, m-phenethyl-	37	034176-91-5	207.27
8	33.008	2.61	2-Nonadecanone, O-methyloxime	60	036379-39-2	311.5
9	33.408	4.13	Nonadecane	95	000629-92-5	268.52
10	33.579	1.96	4'-Benzyl-2'-hydroxy-6'-methyl-3'-phenylacetophenone	49	064648-09-5	438.5
11	33.813	5.07	3.beta.-Acetoxy-17-methyl-5.alpha.-18(13-17)abeoandrost-13-ene	83	072166-08-6	332.5
12	33.968	3.62	1a,9b-dihydro-4-methyl-1H-phenanthro[9,10-b]azirine	83	111005-47-1	362.3
13	34.145	8.31	1,1-DICYANO-2-METHYL-4-(P-CYANOPHENYL)PROPENE	83	000000-00-0	207.23
14	34.383	3.36	1H-Indole, 2-methyl-3-phenyl-	80	004757-69-1	207.27
15	34.669	3.42	Nonadecane, 9-methyl-	95	013287-24-6	282.5
16	35.888	2.83	Eicosane	96	000112-95-8	282.55

Table 2: GC MS analysis of *Penicillium italicum* solid state fermentation filtrate for chemical compounds detection

No	RT (min)	Area%	Name	Quality	CAS Number	M.W
1	10.749	0.35	.BETA. FENCHYL ALCOHOL	90	000470-08-6	154.25
2	13.81	0.27	.beta.-Myrcene	42	000123-35-3	136.23
3	17.333	0.43	Valencene	99	004630-07-3	204.35
4	17.686	0.66	4-Methyl-2,6-di-tert-butylphenol	98	000128-37-0	250.38
5	25.148	0.76	Methyl palmitate	97	000112-39-0	270.45
6	25.926	0.30	E-11-Hexadecenoic acid, ethyl ester	53	000000-00-0	282.5
7	26.227	2.19	Hexadecanoic acid, ethyl ester	99	000628-97-7	284.47
8	27.778	0.69	8,11-Octadecadienoic acid, methyl ester	99	056599-58-7	294.5
9	27.877	0.76	Methyl trans-8-octadecenoate	99	026528-50-7	296.5
10	28.281	0.28	Methyl stearate	98	000112-61-8	298.5
11	28.78	2.40	Ethyl linoleate	99	000544-35-4	308.5
12	28.873	1.43	Ethyl Oleate	98	000111-62-6	310.51
13	29.314	0.28	Docosane	62	000629-97-0	310.602
14	30.736	0.46	Tricosane	96	000638-67-5	324.63
15	32.095	0.30	Tetracosane	98	000646-31-1	338.65
16	32.759	0.50	Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-	37	022606-97-9	251.4
17	33.408	0.48	Nonadecane, 9-methyl-	93	013287-24-6	282.5
18	33.813	0.79	3.beta.-Acetoxy-17-methyl-5.alpha.-18(13-17)abeoandrost-13-ene	90	072166-08-6	332.5
19	34.103	70.34	Diisooctyl phthalate	91	027554-26-3	390.6
20	37.419	0.30	Squalene	93	007683-64-9	410.7
21	40.392	0.29	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	43	019095-24-0	577.2
22	41.736	0.68	24-methylcholesta-5,7,24(28)-trien-3.beta.-ol	38	023582-83-4	396.6
23	41.959	1.16	ERGOST-5-EN-3.BETA.-OL	52	004651-51-8	400.7
24	42.395	0.45	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	49	019095-24-0	577.2
25	43.199	6.63	Stigmasterol, 22,23-dihydro-	99	000000-00-0	412.7
26	43.489	0.25	1,1,1,3,5,5,5-Heptamethyltrisiloxane	42	001873-88-7	221.5
27	43.77	1.85	Bis(methyloxmine), monotrimethylsilyl-6.alpha.-Hydroxyandrostenedione	86	000000-00-0	302.4
28	44.019	3.27	3',4',5,6,7,8-Hexamethoxyflavone	91	000478-01-3	418.4
29	44.413	0.29	Demecolceine	43	000518-11-6	371.4
30	45.404	0.56	Testosterone Cypionate	47	000058-20-8	412.61
31	48.865	0.31	N-Methyl-1-adamantaneacetamide	42	000000-00-0	207.31

The results of the current study shown in figure 4 appeared the chloroformic extracts activity of *P. italicum* SSFF and LFF against pathogenic bacteria. Table 3 explains the significant differences between

them at $p < 0.001, 0.02, 0.05$ and 0.06 , with IZ: $24.7 \pm 0.57, 18.2 \pm 0.28, 26.3 \pm 0.59, 21.6 \pm 0.51$ and 32.8 ± 0.21 (for SSFF) mm respectively and $0.0, 12.3 \pm 0.57, 28.16 \pm 0.20, 19.3 \pm 1.15$ and 28 ± 0.2 (for LFF) mm respectively.

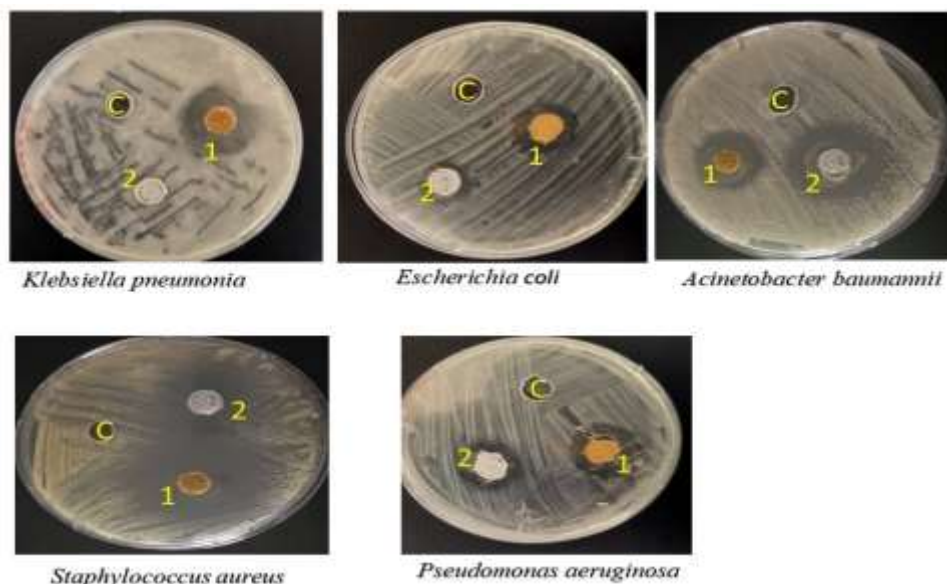


Figure 4:

Effect of 50 mg mL^{-1} of *Penicillium italicum* filtrates on pathogenic bacteria measured by the diameter of the inhibition zone (mm): 1, Solid state fermentation filtrate 2, Liquid fermentation filtrate C, Control (DMSO 10%).

Table 3: Effect of 50 mg mL^{-1} of SSFF and LFF on pathogenic bacteria measured by the mean diameter of the inhibition zone \pm Standard deviation (mm)

Name of bacteria	SSF Filtrate (mean \pm SD)	LF Filtrate (mean \pm SD)	P value	Control			P value
<i>Klebsiella pneumoniae</i>	24.7 ± 0.57	0.00	$p < 0.001$	0.00	0.00	0.00	$p < 0.001$
<i>Escherichia coli</i>	18.2 ± 0.28	12.3 ± 0.57	$p < 0.05$	0.00	0.00	0.00	$p < 0.001$
<i>Acinetobacter baumannii</i>	26.3 ± 0.59	28.16 ± 0.20	$p < 0.06$	0.00	0.00	0.00	$p < 0.001$
<i>Pseudomonas aeruginosa</i>	21.6 ± 0.51	19.3 ± 1.15	$p < 0.01$	0.00	0.00	0.00	$p < 0.001$
<i>Staphylococcus aureus</i>	32.8 ± 0.21	28 ± 0.2	$p < 0.02$	0.00	0.00	0.00	$p < 0.001$

SSFF: Solid State Fermentation Filtrate

LFF: Liquid Fermentation Filtrate

SD: standard deviation

P: Probability of significant difference

Discussion

Upon culturing on SDA, colonies of mold appear fast growing; the colony size of *P. italicum* can reach 5cm within a week when grown on CZA at 28 ± 2 °C, but quickly assumes a greenish-blue pigmentation due to abundant conidium formation; the pigmentation of mature conidia is at least partly due to melanin (6). Microscopic examination showed the conidiophores, which are terverticillate, hyaline metulae that are more or less cylindrical, smooth-walled, and bear three to six phialides each, originate from the substratum or occasionally from superficial hyphae. The asymmetric penicilli bearing tangled chains of conidia make up the conidial apparatus. The phialides have short, distinct necks and are cylindrical and narrow. Conidia are $4.0\text{--}5.0 \times 2.5\text{--}3.5$ μm in size, smooth, greenish, and have smooth walls. Phialides can be generated single, in groups, or from branched metulae, giving the appearance of a brush (a penicillus) (18,19). Figure 1 shows the macroscopic and microscopic characteristics of this mold. The amount of the extract depends on the nature of the culture medium for mold growth, where the orange is a nutrient-rich material (20), Moreover, the secondary metabolites that this mold produces are known as virulence factors, and it is well established that these substances both promote the development of disease and impede or inhibit the fruit's defense mechanism in various pathogen-host interactions (2) and this explains the increased amount of mold filtrate growing on the infected fruit as solid-state fermentation. While YES medium is a medium that contains only two components, yeast extract and sucrose(15).

Through chemical analysis of *P. italicum* filtrates under consideration, it was found that they contain

many compounds that are biologically effective, especially against microorganisms, sixteen bioactive compounds were identified in the chloroformic extract of *P. italicum* in liquid fermentation filtrate LFF and thirty-one compounds were detected in the extract of solid-state fermentation filtrate SSFF (tables 1 and 2). The identification of bioactive chemical compounds is based on the peak area, retention time, molecular weight and chemical structure. Several previous studies were conducted on the chemical content of secondary metabolites for this mold filtrate (21). In a study performed by Mohammed et al (8) of liquid fermentations, they found that twenty-eight bioactive chemical constituents were identified by (GC-MS) from methanolic extract of the *P. italicum* in liquid fermentation by potato dextrose broth (PDB) medium and the main composite was decanoic acid and its derivatives. A chemical study with The FTIR analysis of *P. italicum* performed by Al Mousawi and Razaq, (22) showed the presence of functional group assignment Alkenes, Alkyl halides, Amide, and Alkane. However, there is no prior study on the solid-state fermentation of this mold in terms of the chemical content of the filtrate. The results of the current study shown in Table 3 appeared that the chloroformic extracts of SSFF and LFF of *P. italicum* against pathogenic bacteria were highly effective in suppressing the growth of gram-positive and gram-negative bacterial species with significant differences between them at $p < 0.001, 0.02, 0.05$ and 0.06 , with IZ: $24.7 \pm 0.57, 18.2 \pm 0.28, 26.3 \pm 0.59, 21.6 \pm 0.51$ and 32.8 ± 0.21 (for SSFF)mm respectively and $0.0, 12.3 \pm 0.57, 28.16 \pm 0.20, 19.3 \pm 1.15$ and 28 ± 0.2 (for LFF)mm respectively. As shown in Figure 4, *Klebsiella pneumonia* was resistant to LFF and the reason may be due to that this bacterium contains a capsule, which is considered a virulence factor that makes it resistant to antibacterial agents, while both filtrates showed the highest efficacy against *Staphylococcus aureus* with significant difference at $p < 0.02$; the reason for

the high efficacy can be attributed to the fact that this bacterium is gram-positive; so the most important causes of resistance in bacteria is the genetic and the environmental factors, and the fact that the patient does not take antibiotics frequently, so the bacteria become sensitive to antibacterial agents (23). In a previous study conducted by Faïd and Tantaoui-Elaraki (24) for sterile filtrates toxigenesis test of twenty-four isolates of *P. italicum* against *Bacillus megaterium*, the filtrates showed toxicity at about 96%. Mohammed et al. (8) demonstrated the antibacterial activity of *P. italicum* volatile chemicals by showing how well they inhibited *Proteus mirabilis* growth at 6.08 ± 0.21 mm. According to Al Mousawi and Razaq (22), this mold was extremely aggressive against *Escherichia coli* (6.02 ± 0.18) mm. The identification of *P. italicum* bioactive chemical products using in vitro antimicrobial determination serves as a foundation for additional phytochemical and pharmacological research aimed at developing novel compounds with potential antibacterial and antifungal properties (8). Investigations into the toxicity of sterile culture filtrates of *Penicillium aurantiogriseum* and *P. viridicatum* against *Bacillus subtilis* were conducted. The effect on *B. subtilis* varied according to the amount of filtrate utilized, and the same study's chemical analysis of the filtrate revealed that it included numerous mycotoxins with cytotoxic activity, including aurantiamine, terrestric acid, and penicillic acid (25). Conclusions: the filtrate of *Penicillium italicum* from a natural medium (rotted orange) as a solid state fermentation was more weighted and gave many effective metabolites compared to what was produced by liquid fermentation on a synthetic medium, and both liquid and solid fermentation filtrates demonstrated efficacy against harmful bacteria.

Recommendations

Study of the effectiveness of blue mold filtrates as antifungal, ant parasite, and anticancer activities.

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Ethical clearance

Official approval has been obtained to use data and data were analyzed without the names to protect privacy. This study was conducted according to the approval of College of Medicine/ University of Diyala and in accordance with the ethical guidelines of the Declaration of ethical committee of the College (Document no. 2024AFH885).

Competing interests

The author declares that they have no conflict of interest.

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النشاط الضد ميكروبي لراشح العفن الأزرق ضد بعض انواع البكتريا المرضية

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الملخص

الخلفية الدراسية: تعد الأنواع الرمية من البنسليوم من بين أشهر الفطريات والتي تعيش بشكل أساسي على المواد العضوية القابلة للتحلل. تضمنت الدراسة الحالية تحضير راشح العفن الأزرق وهو عفن نباتي رمي شائع في يهاجم النباتات ما بعد الحصاد يرتبط عادةً بالحمضيات.

الهدف من الدراسة: استهدفت الدراسة تحديد الوسط الأكفأ لنمو العفن وإنتاج نواتج الأيض الثانوي المضادة للبكتريا.

طرق العمل: شملت الدراسة تحديد وسط النمو للعفن الأزرق وإنتاج مواد الأيض الثانوي له من خلال استخدام تقنية كروماتوغرافيا الغازية مطياف الكتلة وتخمر الحالة السائلة لكل من راشح تخمر الحالة الصلبة وتخمر الحالة السائلة بالإضافة الى ذلك، تم تقييم النشاط الضد ميكروبي لراشحي العفن ضد البكتريا المرضية باستخدام طريقة الانتشار من الحفر.

النتائج: أوضحت النتائج أن الكتلة الحيوية لراشح العفن كانت أكبر كمية في حالة تخمر الحالة الصلبة مقارنة بتخمر الحالة السائلة. كان وزن المستخلص الخام من مكررين من الوسط السائل ٠,٠٦ ملغم ٠,١١ ملغم والمستخلص الخام من البرتقال المتعفن كان ملغم أظهرت نتائج الكروماتوغرافيا الغازية أن راشحي العفن يحتويان على عدة مركبات معروفة بفعاليتها الطبية مثل التيتراكونان . عموماً أظهر كلا الراشحين فعالية تثبيته ضد البكتريا المرضية وبأقطار تثبيته تمثلت بـ:

$$٠,٥٧ \pm ٢٤,٧, ٠,٢٨ \pm ١٨,٢, ٢٦,٣ \pm ٠,٥٩, ٢١,٦ \pm ٠,٥١, ٣٢,٨ \pm ٠,٢١$$

لراشح تخمر الحالة الصلبة مقاساً بالملم على التوالي.

وبأقطار تثبيته تمثلت بـ :

$$١٠,٠٧ \pm ١٢,٣, ١٠,٢٠ \pm ٢٨,١٦, ١٠,١٥ \pm ١٩,٣, ١٠,١٥ \pm ١٩,٣, ٢٨ \pm 0.2 \text{ and}$$

لراشح تخمر الحالة السائلة مقاساً بالملم على التوالي

الاستنتاجات: كان لراشح العفن الأزرق المستخلص من البرتقال المتعفن وزن أكبر وكمية مواد ايض ثانوي أكثر مقارنة براشح العفن المستخلص من تخمر الحالة السائلة فيما اظهر كلا النوعين من الراشح الفعالية المضادة للبكتريا المرضية

الكلمات المفتاحية: العفن الأزرق، راشح تخمرات الحالة الصلبة، راشح تخمرات الحالة السائلة الكروماتوغرافيا الغازية، النشاط المضاد للبكتريا

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