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The inhibitory activity of extracts of some edible fungi on the growth of two types of bacteria pathogenic to humans

Mallak Mahlan. Ammar

<https://orcid.org/0000-0001-5010-4375>

malak.m83@uomustansiriyah.edu.iq

Science Department, College of Basic Education, Al-Mustansiriya University, Baghdad, Iraq.

Abstract:

This study was conducted praise be to God, in the laboratories of the Science Department of the College of Basic Education- Al-Mustansiya University- for the period from 7/1/2025 to 17/2/2025. This study was conducted to study the inhibitory activity of chloroform, 70% ethanol, and hot water extracts of the fruiting bodies of some edible fungi, namely, Lion's Mane (*Hericium erinaceus*), Enoki (*Flammulina velutipes*), and *Cantharellus cibarius*, at the following concentrations: 25, 50, and 100 mg/ml, against two pathogenic bacterial species, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This was done using the drill diffusion method. The three extracts showed a remarkable inhibitory activity against the two types of pathogenic bacteria under study. This activity increased with increasing concentration. The highest effect was for the 70% ethanol extracts at a concentration of 100 mg/ml against Gram-positive *Staphylococcus aureus*, as the diameter of the inhibition zones reached (20, 19, 17) mm, followed by the chloroform extracts, which reached (19, 18, 16) mm, and then the hot water extracts, which were the least (20, 17, 12) mm for the fungi Lion's Mane, *Cantharellus aureus*, and Enoki, respectively. The effect of these extracts on the Gram-negative *Pseudomonas aeruginosa* was recorded to a lesser degree, as the diameters of the inhibition zones reached (19, 17, 12.5) mm, (15, 17, 12) mm, and (14, 16.5, 9) mm for the three extracts and fungi, respectively. In conclusion, I would like to extend my sincere thanks to Al-

Mustansiriya University for all the facilities, it provided to prepare this promising scientific research, to achieve some of the goals of sustainable development.

Keywords: Inhibitory ;activity; lion's mane; extracts; *Cantharellus ;aureus*; enoki ‘ edible mushrooms

respectively. The lion's mane fungus has a long history of use in traditional Asian medicine, as it contains compounds such as erinacin and hericin, which have antibacterial and anti-inflammatory effects (6). The enoki fungus is rich in polysaccharides and active proteins. Bioactive, it has been proven effective as a natural antimicrobial (7). *Cantharellus aureus* also contains phenolic compounds and antioxidants that have shown inhibitory effects on several types of pathogenic bacteria (8). *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause many human diseases and are known targets in recent laboratory studies (9). Fungal extracts, such as those taken from *Schizophyllum commune*, *Hypholoma fasciculare*, *Suillus collitinus*, and others, have shown a clear ability to inhibit their growth and reduce biofilm formation. This suggests that some edible fungus may be promising options as environmentally sustainable antibacterial agents, both Gram+ and Gram-. Therefore, the current study aimed to evaluate the inhibitory activity. Extracts of some edible fungi, namely Lion's Mane (*Hericium erinaceus*), Enoki (*Flammulina velutipes*) and *Cantharellus cibarius*, were shown to inhibit the growth of two human pathogenic bacteria,

Introduction:

There has been a growing interest in finding natural alternatives to chemical antibiotics in recent times. This is due to the growing resistance of microorganisms (especially bacteria) to these traditional antibiotics, in addition to the negative side effects resulting from the long-term use of chemical drugs (1). Furthermore, there is a growing awareness and desire among individuals to adopt new lifestyles based on alternative natural therapeutic sources that are both safe and effective, as well as environmentally sustainable compared to synthetic drugs (2). In this field, medicinal plants, edible fungi, and other natural materials have emerged as rich sources of bioactive compounds that possess numerous therapeutic properties, including antibacterial, antioxidant, anti-inflammatory, and even anticancer properties (3). They are effective against a wide range of human pathogenic microorganisms because they contain numerous phenolic compounds, terpenoids, polysaccharides, and other bioactive compounds (4, 5). Among these fungi are the lion's mane (*Hericium erinaceus*), the enoki fungus (*Flammulina velutipes*), and the golden cantharellus fungus (*Cantharellus cibarius*) Figure (1, 2, 3)

efficacy of natural extracts of these fungi is a promising step towards developing alternative, sustainable and environmentally friendly antimicrobials.

Staphylococcus aureus and *Pseudomonas aeruginosa*. Given the global challenges related to the increasing resistance of these strains to antibiotics (10), exploring the



Figure (1) The external appearance of the lion's mane mushroom



(Figure (2) The external appearance of the golden cantharellus mushroom (*Cantharellus cibarius*)



Figure (3) The external appearance of the enoki mushroom (*Flammulina velutipes*)

Research Laboratory of the College of Basic Education/Al-Mustansiriya University, according to the instructions of the supplying companies. All media were sterilized in an autoclave at 121°C and 15 psi for 15 minutes (11).

Preparation of Bacterial Suspension:

Bacterial suspension was prepared at a concentration of 1.5×10^8 cells/ml by transferring 4-5 isolated colonies growing on their solid media to test tubes containing 10 ml of Mueller-Hinton Broth. The tubes were incubated at 37°C for 5-6 hours until turbidity appeared. This turbidity was compared with the 0.5 McFarland tube suspension (standard suspension) (9).

Preparation of Fungal Extracts:

Extracts of the edible fungi under study were prepared according to the method of (10), with minor modifications using chloroform, 70% ethanol, and hot water. 100 grams of each fungus powder was transferred to three

Materials and Methods:

Source of organisms used in the study:

Dried fruiting bodies of edible fungi were obtained from Shaanxi Y Herb Biotechnology Co. Ltd., People's Republic of China. The fruiting bodies were ground in an electric grinder and stored in opaque bags until use. All pathogenic bacterial isolates were identified from pathological samples received at Al-Yarmouk Teaching Hospital in Baghdad, Iraq. Two bacterial isolates were identified, one of which was Gram-positive (*Staphylococcus aureus*) and one Gram-negative (*Pseudomonas aeruginosa*).

Cultivation media used:

- Broth nutrient medium for the growth and activation of bacterial isolates.
- Mueller-Hinton agar medium: used to study the antibacterial activity of the fungal extract using the hole diffusion method. All media were prepared in the Graduate Studies

diffuser. The plates were left to dry. 5-mm-diameter holes were then made on the surface of the solid culture medium using a sterile cork drill. The prepared extract concentrations were transferred to Drilling holes with a volume of 50 μ L into each hole. The plates were incubated at 37°C for 24 hours, and the effectiveness of the extract was determined by measuring the diameter of the inhibition zone around the holes, measured in millimeters (12).

Statistical Analysis:

SAS software was used to statistically analyze the results obtained when studying the effect of different concentrations of the three edible fungal extracts against pathogenic bacteria. Significant differences between the means were compared using the least significant difference (LSD) test ($p \leq 0.05$). Four treatments were performed, each with two replicates (13).

Results and Discussion:

Table (1) shows the inhibitory activity of lion's mane (*Hericium erinaceus*) fungus extracts against two types of bacteria: *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This activity was performed using three solvents (chloroform, 70% ethanol, and hot water) and three different concentrations (25, 50, and 100 mg/ml). The average diameter of the inhibition zone (in mm) was measured using a solid-state diffusion test.

separate conical flasks. The first three flasks were mixed with 1 liter of chloroform (99.8%), the second three flasks with 70% ethanol, and the third three flasks with hot distilled water heated to 60°C and incubated for 2 hours at 25°C. The flasks were shaken for 72 hours at 150 rpm using an incubator shaker. The extracts were centrifuged at 3000 rpm for 15 minutes, filtered using Whatman No. 1 filter paper, and then concentrated and dried in a rotary evaporator at 50°C. The extracts were frozen in an MRC FDL-10N-50-8M freeze dryer at -80°C, and then freeze-dried. Finally, the crude extracts were stored in a refrigerator at 4°C in opaque bottles for further use. Solutions were then prepared at concentrations of 100, 50, 25, and 12.5 mg/ml.

Study of the effect of the three edible fungal extracts under study on the growth of pathogenic bacterial isolates:

The drill diffusion method was used to study the effect of chloroform, 70% ethanol, and hot water extracts of the edible fungi, namely, lion's mane (*Hericium erinaceus*), enoki fungus (*Flammulina velutipes*), and *cantharellus cibarius*, on the human-pathogenic bacterial isolates, namely, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This was done by transferring 0.1 ml of the bacterial suspension, containing 1.5×10^8 cells/ml, to plates containing Mueller-Hinton agar medium and spreading it over the surface of the medium using a sterile glass

inhibits bacterial growth, disrupts the internal enzymes of pathogenic bacteria, and inhibits intracellular energy production (15). The hot water extract was somewhat surprising in its effectiveness, especially at a concentration of 100 mg/ml, when it was close to that of ethanol. This is attributed to the extraction of water-soluble polysaccharides such as β -glucans and hericenones, which are known for their immunomodulatory and antibacterial roles (11). Meanwhile, the chloroform extract showed a relatively lower effect, especially against Gram-negative bacteria. This is expected because chloroform extracts non-polar compounds, which are often less effective on bacteria than phenols or polysaccharides. Therefore, it can be said that *Herichium erinaceus* extracts have promising activity as natural antibacterial agents. 70% ethanol is considered the best solvent for extracting active antibacterial compounds. These results are consistent with previous studies on the inhibitory efficacy of medicinal fungi and support the use of fungus as a natural alternative source of antimicrobial agents.

All extracts demonstrated a clear inhibitory activity that increased with increasing extract concentration. This is consistent with the results of previous studies confirming a direct relationship between extract concentration and inhibition zone diameter (14).

In general, the extracts were more effective against *Staphylococcus aureus* (Gram-positive) than *Pseudomonas aeruginosa* (Gram-negative). This is probably due to the fact that the cell wall of negative bacteria contains a lipopolysaccharide layer that hinders the entry of active compounds (19). It is also noted from Table (1) that at a concentration of 100 mg/ml, the 70% ethanol extract was the most effective against both species, reaching 20 mm against *Staphylococcus aureus* and 19 mm against *Pseudomonas aeruginosa*, followed by the hot water extract and then chloroform. The superiority of 70% ethanol may be due to its ability to extract polar and semi-polar compounds such as phenols, terpenes, flavonoids, and aromatic compounds. These compounds are known for their antibacterial effect by damaging the cell wall. It also

Table (1) Inhibitory efficacy of extracts of the lion's mane fungus (*Herichium erinaceus*) against some pathogenic bacteria:

Type of bacteria	Lion's Mane (<i>Herichium erinaceus</i>) extracts			values LSD
	Average diameter of the inhibition zone in mm according to the (extract concentration (mg/ml			
	Chloroform	70% ethanol	Hot water	

	25	50	100	25	50	100	25	50	100	
<i>Staphylococcus aureus</i> (+)	8	15	19	9	16	20	7	15	20	4.59
<i>Pseudomonas aeruginosa</i> (-)	6	13	15	7	14	19	6	12	14	3.99

methanolic/ethanolic extract of *Cantharellus cibarius* showed higher activity against Gram-positive bacteria than Gram-negative bacteria, recording an inhibition zone of up to 21 mm. This confirms that organic solvents extract the most active antibacterial compounds. (12) showed that methanol showed greater activity than ethanol, but the use of 70% ethanol was very effective. As for chloroform, (18) stated in their study as part of the efficacy tests that the chloroform extract was sometimes more effective against bacteria and fungi than ethanol and water, using the diffusion method, but it is less stable and safe for human use. As for the hot water extracts of *Cantharellus cibarius*, many studies have shown its antioxidant activity, which may be due to pure compounds, but it is usually less than organic extracts (18).

Table (2) shows the inhibitory activity of *Cantharellus cibarius* extracts against some pathogenic bacteria. It shows that the best sustained activity and inhibition capacity per milliliter was recorded by the 70% ethanol extract, which showed the highest values at a concentration of 100 mg/ml, reaching 19 mm, surpassing chloroform (18 mm) and hot water (17 mm). Chloroform slightly outperformed at a concentration of 25 mg/ml, reaching 6.5 mm, compared to hot water (6.15 mm) and ethanol (5.7 mm) at the same concentration. At concentrations of 50 and 100 mg/ml, 70% ethanol consistently outperformed, reaching 13 and 19 mm, followed by chloroform, which reached 12 and 18 mm. It appears that the 70% ethanol extract was the most sensitive against the two types of bacteria under study. These results are consistent with the findings of (16) that the

Table (2) Inhibitory activity of extracts of the golden *Cantharellus* (*Cantharellus cibarius*) fungus against some types of pathogenic bacteria.

Type of bacteria	Cantharellus cibarius extract (<i>Cantharellus cibarius</i>)			values
	Average diameter of the inhibition zone in mm according to the extract concentration (mg/ml)			LSD
	Chloroform	Ethanol 70%,	Hot water	

	25	50	100	25	50	100	25	50	100	
<i>Staphylococcus aureus</i>	7	12	18	5.9	13	19	6.4	13.5	17	11.8
<i>Pseudomonas aeruginosa</i>	6	13	17	5.5	12	17	5.9	12.5	16.5	13.7

top to bottom with ethanol, then chloroform, followed by hot water. It was also noted that Gram-positive bacteria (*Staphylococcus aureus*) were more sensitive than Gram-negative bacteria (*Pseudomonas aeruginosa*) across all extracts. This may be due to the difference in cell wall permeability of Gram-positive bacteria, which have a thinner and less complex cell wall compared to Gram-negative bacteria, making them more susceptible to the bioactive compounds present in fungal extracts (19). Ethanol and chloroform are organic extracts that extract lipid compounds such as phenols, terpenoids, and glycolipids, which are known for their bioactivity and bactericidal activity against pathogenic bacteria, while water extracts only polar compounds such as sugars and phenols, which are less effective against the bacteria themselves. This leads us to think that the increase in the diameter of the inhibition zone with increasing concentration suggests that beneficial compounds are present at higher concentrations in Ethanol and chloroform extracts. This is consistent with the results of numerous studies showing that 75–95% ethanol extracts yield inhibition angles

Table (3) showed that the enoki fungus (*Flammulina velutipes*) extract decreased compared to the lion's mane (*Hericium erinaceus*) and *cantharellus cibarius* extracts. The chloroform extract recorded an increase in the average diameter of the inhibition zone in *Staphylococcus aureus* bacteria, reaching (9, 13 and 16 mm). It recorded (7.1, 9.3 and 12 mm) in *Pseudomonas aeruginosa*. At concentrations of (25, 50 and 100 mg/ml (respectively), the 70% ethanol extract recorded an average diameter of the inhibition zone for *Staphylococcus aureus* and *Pseudomonas aeruginosa* of (9.5, 13.9 and 17 mm) and (7.3, 9.1 and 12.5 mm) at concentrations of (25, 50 and 100 mg/ml (respectively). As for the hot water extract of enoki fungus, the average diameter of the inhibition zone for *Staphylococcus aureus* and *Pseudomonas aeruginosa* was (5, 11 and 12 mm) and (4, 7 and 9 mm) at concentrations of (25, 50 and 100 mg/ml) respectively. It appears that the antibacterial activity is related to the concentration of the extract, i.e., the inhibition increases with increasing concentration. Accordingly, the efficiency of the fungus extracts can be arranged. Enoki (*Flammulina velutipes*) were incubated from

and bacteria (20).

between 9–36 mm, depending on the species

Table (3) Inhibitory activity of enoki fungus (*Flammulina velutipes*) extracts against some pathogenic bacteria:

Type of bacteria	Enoki fungus extract (<i>Flammulina velutipes</i>)									values LSD
	Average diameter of the inhibition zone in mm according to the extract concentration (mg/ml)									
	Chloroform			70% ethanol			Hot water			
	25	50	100	25	50	100	25	50	100	
<i>Staphylococcus aureus</i>	9	13	16	9.5	13.9	17	5	11	12	11.5
<i>Pseudomonas aeruginosa</i>	7.1	9.3	12	7.3	9.1	12.5	4	7	9	8.9

aeruginosa, the 70% ethanol extract of the Lion's Mane fungus clearly outperformed, with an inhibition zone diameter of 19 mm, compared to (15 and 14) mm for chloroform and hot water, respectively. Since the differences here (4 and 5 mm) exceeded the LSD value, they are considered statistically significant differences, indicating that the 70% ethanol extract is the most effective against this bacteria. Studies have supported This result is preceded by the fact that lion's mane fungus contains bioactive compounds such as erinacines and hericenones, which are characterized by their antimicrobial properties (21,22). *Cantharellus cibarius* showed moderate activity, with a maximum inhibition zone diameter of 19 mm against *Staphylococcus aureus* using a 70% ethanol extract. Enoki fungus (*Flammulina velutipes*)

As for Table No. 4, its results showed that the extracts taken from the lion's mane fungus (*Hericium erinaceus*) achieved the highest antibacterial effectiveness compared to the rest of the types of fungal extracts under study, at a concentration of (100 mg/ml). The 70% ethanol extract of this fungus recorded the largest inhibition zone diameter against *Staphylococcus aureus*, reaching 20 mm, which is the same value achieved by the hot water extract. However, the difference between the two extracts is not considered statistically significant, while the difference between the ethanol and chloroform extracts was (1 mm), which is also less than the estimated LSD value of 2 mm, indicating that there are no real significant differences between these extracts against *Staphylococcus aureus*. As for *Pseudomonas*

water. This is consistent with the findings of (32) and (25), which confirm that ethanol is very effective in extracting phenolic and terpenic compounds with high biological activity. Therefore, it can be said that the 70% ethanol extract of Lion's Mane fungus is a promising candidate for the development of natural antibiotics, especially effective natural antibacterials, against strains resistant to chemical antibiotics, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

showed less activity against *Pseudomonas aeruginosa*, with an inhibition zone diameter of 12.5 mm and a decrease to 9 mm with the aqueous extract. This indicates the weak biological activity of this type of extract. These results are consistent with the study by (23). These results also showed that the effectiveness of the extracts varies depending on the fungus species and the solvent used, with the extract of 70% ethanol was found to be the most effective in extracting the active compounds compared to chloroform and hot

Table (4) Effect of fungal extracts at the optimum concentration (100 mg/ml) on different types of bacteria.

Type of fungus	Type of bacteria	Type of extract at best concentration (100 mg/ml)	Maximum inhibition zone diameter
(Hericium erinaceus)	<i>Staphylococcus aureus</i>	Chloroform	^a 19
		Ethanol 70%	^a 20
		Hot water	20 ^a
	<i>Pseudomonas aeruginosa</i>	Chloroform	15 ^b
		Ethanol 70%	19 ^a
		Hot water	14 ^b
(Cantharellus cibarius)	<i>Staphylococcus aureus</i>	Chloroform	18 ^a
		Ethanol 70%	19 ^a
		Hot water	17 ^a
	<i>Pseudomonas</i>	Chloroform	17 ^a

	<i>aeruginosa</i>	Ethanol 70%	17 ^a
		Hot water	16.5 ^a
(Flammulina velutipes)	<i>Staphylococcus aureus</i>	Chloroform	16 ^a
		Ethanol 70%	17 ^a
		Hot water	12 ^b
	<i>Pseudomonas aeruginosa</i>	Chloroform	12 ^b
		Ethanol 70%	12.5 ^b
		Hot water	9 ^c

LSD values at P < 0.05 are 2.0. Symbols a, b, c within each bacterial group. Similar letters mean no significant differences. Different letters indicate significant differences when LSD values ≥ 2.

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