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ANTIBACTERIAL ACTIVITY OF *AGARICUS BISPORUS* AND *PLEUROTUS OSTREATUS* EXTRACTS AGAINST SOME GRAM NEGATIVE AND POSITIVE BACTERIA

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ABSTRACT
The present study focused on the evaluation of *Agaricus bisporus* and *Pleurotus ostreatus* activity against two clinical isolates *Escherichia coli* and *Staphylococcus aureus*. The cold water and hot water extract of *Agaricus bisporus* and *Pleurotus ostreatus* showed maximum inhibition at 75% concentration against *S. aureus* and *E. cole* respectively. Ethanol, chloroform and acetone mushroom extracts of *Agaricus bisporus* and *Pleurotus ostreatus* at the highest concentration (75%) caused highest reduction of growth for *S. aureus* and *E. cole.*

KEYWORDS: *Agaricus bisporus*, *Pleurotus ostreatus*, Antimicrobial.

INTRODUCTION
Basidiomycota include mushrooms. There are about 14,000 mushroom species. Only 10% are well known.[1] Mushrooms used in different parts of the world for centuries as medicines against many diseases. Anticancer, anti-viral and antitumor compounds have been found in mushroom.[2] Mushrooms also used as immunomodulatory, cardiovascular, liver protective, anti-fibrotic, antiinflammatory, anti-diabetic, antioxidant and antimicrobial properties.[3] Quinine, also commonly found in mushrooms, was used in the first century for the treatment of tuberculosis.[4] High levels of antioxidant compounds such as ascorbic acid, phenols, and tocopherols were found in *Agrocybe cylindracea* using hot water extraction methods.[5] *Trichloma giganteum* was also explored for antitumor activity.[6] The genus *Pleurotus* including several cultivated species such as *P. ostreatus, P. Pulmonary, P. Cornucopia, P. eryngii* and *P. cystidiosus*.[7] Approximately 70 species of *Pleurotus* have been recorded. Many of these species exhibit antimicrobial properties.[8] Mushroom extracts having antimicrobial activity against Gram-positive bacteria. Using a methanolic extract of *Agaricus bisporus* revealed MIC = 5µg/mL against *Bacillus subtilis*.[9] and also showed activity against *Bacillus cereus, Micrococcus luteus, Micrococcus flavus, Staphylococcus aureus* and *S. epidermidis*.[10] *Agaricus bitorquis* methanolic extracts showed inhibitory effect upon all the tested Gram-positive bacteria, *Agaricus silvicola* methanolic extract also revealed antimicrobial properties against *Bacillus cereus* (MIC = 5µg/mL), *Bacillus subtilis* (MIC = 50µg/mL), and against *Staphylococcus aureus* (MIC = 5µg/mL).[11] *Pleurotus ostreatus* showed broad-spectrum antimicrobial activity. The maximum effect was shown by ethanolic extracts of *Pleurotus ostreatus* against *Sarcina lutea*.[12] *Agaricus bisporus* and *Pleurotus ostreatus* are the most cultivated mushroom and accounts for the 38% of worlds cultivated mushrooms. Therefore, the present study focused on the evaluation of antibacterial activities of hot water, cold water, ethanol, chloroform and acetone extracts of *Agaricus bisporus and Pleurotus ostreatus* using the agar well diffusion method against two clinical isolates *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS
Collection of Mushroom samples
Tow species of mushrooms, *Agaricus bisporus* (were collected from local markets placed in sterile Polyethylene bags and kept at a temperature of 4 ºC until use) and *Pleurotus ostreatus* (were brought from Al-Qadisiyah University). The material was brought to the laboratory and preserved at room temperature.

Microbial Pathogens used
The bacteria used in this experiment are one Gram-positive bacteria: *Staphylococcus aureus* and one Gram-negative bacteria: *Escherichia coli* were collected from the Microbiology Laboratory by Al- Nahrain university, College of Biotechnology.

Strains	Source
Staphylococcus aureus	Al-Nahrain university / College of Biotechnology
Escherichia coli	

Water Mushroom Extracts Preparation

Fresh whole mushrooms (50 g) were ground using a blender (Singsung - Singapore) and extracted sequentially by soaking twice with 250 ml of of hot water, cold water, for 48 hrs. The extracts were filtered using a filter paper and further concentrated in a vacuum evaporator. The concentrates were then stored in 4° C for further passes. The extracts were collected and stock solution of concentration 10 mg/ml was prepared and were different concentrations (25%, 50% and 75%).[13]

Preparation of Mushroom alcoholic extracts

The fresh fruiting bodies were dried in shade conditions and the dried material (50 g) was pulverized in a blender to get a coarse powder and soaked separately in 200 ml ethanol, chloroform and acetone in flask for extracts. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction.

These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using a rotary evaporator.[14-15] The extracts were collected and stock solution of concentration 10 mg/ml was prepared.

Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The ethanol, chloroform and acetone extracts of *Agaricus bisporus* and *Pleurotus ostreatus* showed considerable growth inhibition of two test bacteria in different concentrations (25%, 50% and 75%).[13]

Screening of extracts of *Agaricus bisporus and Pleurotus ostreatus* for antibacterial activity

Screening of mushroom extracts (ethanol, chloroform and acetone) of *Agaricus bisporus* and *Pleurotus ostreatus* was using agar well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled Water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121°C for 20 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µl of bacterial suspension was spread on each nutrient agar plate. Five agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plot were loaded with 25%, 50% and 75% concentration of prepared extracts of *A. Bosporus* and *P. ostreatus.* The control well containing pure solvent only. The plates were incubated at 37 ± 20C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard.[16]

% growth inhibition = Control - Test / Control x 100
Control= average diameter of bacterial colony in control.
Test = average diameter of bacterial colony in treatment sets.[17]

RESULTS AND DISCUSSION

Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* against *S. aureus* and *E. cole* uses cold water extraction

The cold water extract of *Agaricus bisporus* and *Pleurotus ostreatus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The cold water extract of *Agaricus bisporus* showed growth inhibition of two species bacteria in different concentrations (25%, 50%, 75%). The cold water extract of *Agaricus bisporus* showed maximum inhibition of 28.8 % and 23.3 % at 75% concentration of the extract against *S. aureus* and *E. coli* respectively, and the cold water extract of *Pleurotus ostreatus* showed maximum inhibition of 31.1 % and 27.7 % at 75% concentration against *S. aureus* and *E.coli* respectively (Table 1). While at 25% concentration against *S. aureus* and *E.coli* by cold extract *Agaricus bisporus* showed growth inhibition 10 % and 6.6 % respectively, the cold extract of *Pleurotus ostreatus* showed maximum inhibition 11.1 % and 8.8 % against *S. aureus* and *E.coli* respectively.

The results that a cold water extract of *Pleurotus ostreatus* showed maximum percent inhibition against *S. aureus* more from *E. coli*. *Pleurotus ostreatus* was more effective than *Agaricus bisporus* against both the bacteria.

Similar studies have evidenced that *P. ostreatus* has a broad spectrum of antimicrobial activity. By using solvents with different polarities[18], found that non-polar solvent extracts of *P. ostreatus* like petroleum ether had a stronger inhibitory activity on both Gram-positive and Gram-negative bacteria but with varying degrees of intensity. These observations are in accordance with the findings of[19], who further validated the antimicrobial potential of *P. ostreatus* and found that "organic solvents consistently displayed better antimicrobial activity than that of the aqueous extract".[19] From the polar solvents, the ethanol extract exhibits the highest activity as found by[20], with a 20 mm inhibition area at 10 mg / ml for *E. coli*.

However, not all bacteria species are sensitive to *L. edodes* extracts, as found by[7], who insensitivity of Bifidobacteria and Lactobacilli to these extracts. The data in this study also confirms another supposition that

as in the case of plant extracts, in the case of mushroom extracts, the Gram-positive bacteria are more susceptible to inhibition as compared to Gram-negative bacteria. This difference in the case of plant extracts was known from numerous previous reports.[21-22]

Table 1: Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of cold water extract of *A. bisporus* and *P. ostreatus.*

No.	Concentration of the cold water extract (%)	Inhibition of growth (%)			
		A. bisporus		*P. ostreatus*	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Control	0.0	0.0	0.0	0.0
2	25	10.0	6.6	11.1	8.8
3	50	17.7	12.2	20	14.4
4	75	28.8	23.3	31.1	27.7

Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* against *S. aureus* and *E. coli* using hot water extract

The hot waterextract of *Agaricus bisporus* and *Pleurotus ostreatus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The hot water extract of *Agaricus bisporus* showed growth inhibition of two species bacteria in different concentrations (25%, 50%, 75%). The hot water extract of *Agaricus bisporus* showed maximum inhibition of 29.9 % and 25.5 % at 75% concentration of the extract against *S. aureus* and *E. coli* respectively, and the hot water extract of *Pleurotus ostreatus* showed maximum inhibition of 33.3 % and 28.8 % at 75% concentration against *S. aureus* and *E.coli* respectively (Table 2).

The results that hot water extract of *Pleurotus ostreatus* showed maximum percent inhibition against *S. aureus* more from *E. coli*. *Pleurotus ostreatus* was more effective than *Agaricus bisporus* against both the bacteria.

The use of antibiotics has reduced the incidence of infectious diseases but their extensive uses in therapy, has led to the appearance of drug-resistant bacteria[23], which is a major public health issue worldwide. For this purpose, numerous plant extracts were screened for antimicrobial properties that could protect people from microbial infections.[24]

The mushroom extracts can also be used in combination with traditional antibiotics, the same way the plant extracts are used. In the literature, there are reports regarding the use of plant crude extracts[25], in combination with fewer amounts of antibiotics for anti-bacterial activities, especially for antibiotic-resistant bacteria, compared to antibiotics alone.[26]

Table 2: Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of hot water extract of *A. bisporus* and *P. ostreatus.*

No.	Concentration of the hot water extract (%)	Inhibition of growth (%)			
		A.bisporus		*P. ostreatus*	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Control	0.0	0.0	0.0	0.0
2	25	11.1	8.8	11.1	9.9
3	50	16.6	12.2	22	15.5
4	75	29.9	25.5	33.3	28.8

Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* against *S. aureus* and *E. coli* using ethanol extract

The ethanol extract of *Agaricus bisporus* and *Pleurotus ostreatus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The ethanol extract of *Agaricus bisporus* showed growth inhibition of two species bacteria in different concentrations (25%, 50%, 75%). The ethanol extract of *Agaricus bisporus* showed maximum inhibition of 29.9 % and 27.7% at 75% concentration of the extract against *S. aureus* and *E. coli* respectively, and the ethanol extract of *Pleurotus ostreatus* showed maximum inhibition of 34.4 % and 30.3 % at 75% concentration against *S. aureus* and *E.coli* respectively (Table 3).

It is evident from the results that ethanol extract of *Pleurotus ostreatus* showed maximum percent inhibition against *S. aureus* more from *E. coli*.3

Pleurotus ostreatus was more effective than *Agaricus bisporus* against both the bacteria. The results of the present study are in agreement with the work of the

earlier workers[27-28], who have also reported strong antibacterial activity of mushroom extract against gram negative bacteria (*E. coli*) and gram-positive (*S. aureus*) bacteria.[29], also reported the antibacterial potential of ethanolic extract of *Pleurotus florida* and *Pleurotus ostreatus.*

Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from mushrooms and distributed worldwide. Mushroom based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts.[30] This study has revealed that the edible mushroom *Agaricus bisporus* exhibited various levels of antimicrobial activity in different solvents.

The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harvested as potential antibacterial substances.

Several external factors have been pointed to explain this fact, such as the heterogeneous enzymatic and oxidative decomposition after collection, different stress conditions associated with each sample, and even dissimilar methodologies applied to phenolic compounds extraction.[31-32]

Table 3: Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of ethanol extract of *A. bisporus* and *P. ostreatus.*

No.	Concentration of the ethanol extract (%)	Inhibition of growth (%)			
		A.bisporus		*P. ostreatus*	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Control	0.0	0.0	0.0	0.0
2	25	12.2	8.8	13.3	10
3	50	17.7	14.4	22	16.6
4	75	29.9	27.7	34.4	30.3

Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* against *S. aureus* and *E. coli* using chloroform extract

The chloroform extract of *Agaricus bisporus* and *Pleurotus ostreatus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg / ml, other concentrations were prepared by serial dilution of stock solution.

The chloroform extract of *Agaricus bisporus* showed growth inhibition of two species bacteria in different concentrations (25%, 50%, 75%). The chloroform extract of *Agaricus bisporus* showed maximum inhibition of 30 % and 25.5 % at 75% concentration of the extract against *S. aureus* and *E. coli* respectively, and the chloroform extract of *Pleurotus ostreatus* showed maximum inhibition of 36.6 % and 33.3 % at 75% concentration against *S. aureus* and *E.coli* respectively (Table 4). While at 25% concentration against *S. aureus* and *E.coli* by chloroform extract *Agaricus bisporus* showed growth inhibition 13.3 % and 10 % respectively, the chloroform extract of *Pleurotus ostreatus* showed maximum inhibition 14.4 % and 11% against *S. aureus* and *E.coli* respectively.

The results that chloroform extract of *Pleurotus ostreatus* showed maximum percent inhibition against *S. aureus* more from *E. coli*. *Pleurotus ostreatus* was more effective than *Agaricus bisporus* against both the bacteria.

Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents.[33] Also observed in this study is that there were variations in the degree of antimicrobial activities of mushrooms. The broad spectrum activity of mushrooms was also brought to light as the extracts of mushrooms showed inhibitory effects on clinical isolates used for this investigation. This suggests that the bioactive products which are contained in mushrooms are in concentrations which exude varying degrees of antimicrobial activity. Furthermore, the study revealed that the bacterial isolates (*Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Finding is in accordance with the findings of.[34-37] It is interesting to note from the results of this study that clinical isolates both Gram positive and Gram negative bacteria were sensitive to the extracts. But the gram positive bacteria showed more sensitivity than gram negative bacteria. This is in collaboration with the findings of.[38] The sensitivity of isolates to the mushrooms and spices extracts implies that intrinsic substance in the extracts is unknown to the microorganisms which made it impossible for them to resist. Also, when the effects of cold water and hot water extracts on the mushrooms and spices are compared with regard to the inhibition of microbial growth, the result showed that the cold water had greater inhibiting effect than the hot water. This is in agreement with the reports of.[39] The results showed that the zones of inhibition exhibited on the agar plates by the mushroom and spices extracts were concentration-dependent. The higher the concentration of the extracts, the larger the zones of inhibition produced.

Table 4: Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of chloroform extract of *A.bisporus* and *Pleurotus ostreatus.*

No.	Concentration of the chloroform extract (%)	Inhibition of growth (%)			
		A.bisporus		*P. ostreatus*	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Control	0.0	0.0	0.0	0.0
2	25	13.3	10	14.4	11
3	50	19.9	14.4	21	17.7
4	75	30.0	25.5	36.6	33.3

Antibacterial activity of *Agaricus bisporus* and *Pleurotus ostreatus* against *S. aureus* and *E. coli* using acetone extract

The acetone extract of *Agaricus bisporus* and *Pleurotus ostreatus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The acetone extract of *Agaricus bisporus* showed growth inhibition of two species bacteria in different concentrations (25%, 50%, 75%). The acetone extract of *Agaricus bisporus* showed maximum inhibition of 28.8 % and 22.2 % at 75% concentration of the extract against *S. aureus* and *E. coli* respectively, and the acetone extract of *Pleurotus ostreatus* showed maximum inhibition of 30 % and 24.4 % at 75% concentration against *S. aureus* and *E.coli* respectively (Table 5). While at 25% concentration against *S. aureus* and *E.coli* by acetoneextract *Agaricus bisporus* showed growth inhibition 9.9 % and 7.7 % respectively, the acetone extract of *Pleurotus ostreatus* showed maximum inhibition 12.2 % and 10% against *S. aureus* and *E.coli* respectively.

The results that acetone extract of *Pleurotus ostreatus* showed maximum percent inhibition against *S. aureus* more from *E. coli*. *Pleurotus ostreatus* was more effective than *Agaricus bisporus* against both the bacteria.

Table 5: Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of acetone extract of *A.bisporus* and *P. ostreatus.*

No.	Concentration of the acetone extract (%)	Inhibition of growth (%)			
		A.bisporus		*P. ostreatus*	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Control	0.0	0.0	0.0	0.0
2	25	9.9	7.7	12.2	10
3	50	18.8	15.5	19.9	17.7
4	75	28.8	22.2	30.0	24.4

Show[10], the ethanolic extracts of *Armillaria melliea* mycelium showed antibacterial effect against *Sarcina lutea*, however, no activity was observed upon other Gram-positive bacteria,[40] reported the antimicrobial ability of several extracts of *Pleurotus sajor-caju against Escherichia coli*, *Enterococcus aerogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were most sensitive to ethanolic, methanolic and xylene extracts.[41] investigated that the extract of Tanzanian *C. cinereus* and reported potential antimicrobial activity against a number of organisms like *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. These new bioactive compounds isolated from these mushroom can be used in the development of new drugs for cure of diseases. Similarly,[42] found that the aqueous extract of *Pleurotus florida* and *Pleurotus aureoviosus* showed the antimicrobial activity against *S. aureus*, *P. aeruginosa* and *Candidia albicans. The* cultivation of *Pleurotus sajorcaju* was done in the laboratory at a small scale on the wheat straw and was used as substrate for maximum production of the mushroom for extraction of bioactive compounds.[43] cultivated *Pleurotus sajor-caju* on three different substrate alone like *Populus deltoides*, *Eupatorium adenophorum*and sericulture waste mixed with paddy straw. It was reported that when *Pleurotus sajor-caju* used alone on these three substrate gave the best result as compare to other. In another study it was found that *Cordyceps militaris* contain a number of bioactive compounds like cordycepin, cordycepic acid, vitamin, adenosine and enzymes etc. The bioactive molecule cordycepin was isolated from *Cordyceps militaris* having a broad spectrum biological activity.[44]

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