In vitro Study on the antimicrobial potentialities of two edible mushrooms (*Agaricus bisporus*) and (*Pleurotus ostreatus*)

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Abstract

The current work was undertaken to evaluate *in vitro* the antimicrobial activity of two edible Mushrooms (Basidiomycetes & macrofungus), *Pleurotus osteratus and Agaricus bisporus* , either fresh or air dried were assyed using aqueous and organic solvents extracts. The assays were done against a panel of standard pathogenic bacteria and fungi. Agar well diffusion assay showed that gram (+ve) bacteria were more sensitive to mushroom extracts than gram (-ve) bacteria especially *Bacillus polymexa*, *Staphylococcus aureus* while in case of fungi, *Aspergillus flavus* & *Fusarium moniliforme* were strongly inhibited by either extracts of mushrooms . Aqueous extracts of either Fresh and dried fruiting bodies of *Pleurotus ostreatus* showed high antimicrobial activity than *Agaricus bisporus* extracts ,The minimum inhibitory concentration (MIC) for the two mushroom extracts were ranged between 20-40 µg/ml for pathogenic bacteria and 30-50 µg/ml for fungi. Thus indicated that the daily intake of mushroom can provide a natural covering isolation of antibiotic to fight against the common pathogenic organisms.

Key words: *Pleurotus osteratus* , *Agaricus bisporus* , Antimicrobial activity MIC , and Mushroom.

1. Introduction

Mushrooms are nutritionally functional foods and represent source of physiologically beneficial and noninvasive medicines. Many pharmaceutical substanceswith potent andunique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide. (**Begell and Wasser, 2003)** Mushroom-based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts. Some of the most recently isolated and identified

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substances from mushrooms have been demonstrated to possess significant antitumor, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective and antidiabetic activities. (**Nitha and Janardhanan, 2007)** The human being benefited from the natural defensive strategies of fungi (the mushroom) that produce antibiotics to fight infections (**Hardman *et al*., 2001 & Tambekar *et al.*, 2006)** Researchers showed antimicrobial activity of several mushrooms **(Gezer *et al.*, 2006; Mercan *et al.*, 2006 and loganathan *et al.*, 2009)**. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases**. (Loganathan *et al*., 2009).**

Therefore, the aim of the present work is to evaluate *in vitro* the antimicrobial potential of fresh and dried mushroom extracts (*Pleurotus osteratus and Agaricus bisporus*) against several microorganisms that have medicinal importance.

2. Materials and methods

2.1 Mushrooms

Two edible mushroom strains tested for their antimicrobial activities. They were belonged to the genus *Pleurotus ostreatus* & *Agaricus bisporus.* They were provided from the Agriculture Research Center, Cairo, Egypt and coded as *No. 268* and *NRRL 2366,* respectively.

2.2 Microorganisms

Eight bacterial strains including some antimicrobial resistance strains and six fungal strains were used as test microorganisms throughout antimicrobial activity testing; all of them were provided from the culture collection of Cairo MERCIN in agriculture faculty, Ain shams university.

2.3 Extraction of active antimicrobial agents

Three different solvents were used for the extraction of active components from the *Pleurotus ostreatus* and *Agaricus bisporus.* The two edible fresh mushrooms were thoroughly washed with clean water, sliced and divided into two parts: One part was air dried at 40 ºC in an oven before analysis and the other part of mushroom was left fresh. Then they were extracted by aqueous and organic solvents (ethanolic & methanolic). This method can be

carried out by **(Tambekar *et al.*, 2006; Jagadish *et al*.2009** & **Nwachukwu and Henrietta, 2010).**

2.4 Assesssment of antimicrobial activity

The following strains of bacteria and fungi were tested : Bacterial strains include eight reference strains: *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10876) ,*Bacillus polymexa (ATCC 8523)Salmonella enteric* (ATCC 14028), *Pseudomonas Aeruginos*(ATCC 2036*a), Bacillus subtilis(* ATCC 6633) and *Klebsiella pneumonia (*ATCC 13883). Fugal strains include six reference strains: *Aspergillus niger*(RCMB 002 007 "2), *Aspergillus flavus (*RCMB 002002"5"),*Penicillium expansum* (RCMB 001001 "1"),*Rhizopus nigricans (*RCMB 049001),*Fusarium moniliform*e (RCMB 008005 "2")and *Candida albicans* (RCMB 005003).

Antimicrobial activity of tested mushroom extracts was determined by the agar well diffusion method .Briefly, the mushroom extracts with a final concentration of 20 mg/mL were sterilized through 0.45-μm membrane filter. Small wells (6 mm in diameter) were made in the agar plates by sterile cork borer. One hundred microliters of the extract (20 mg/mL) of each isolate of mushrooms was loaded into the different wells

The concentration of the target bacterial cells suspensions was adjusted to 10^4 cfu/mL. In order to determine the antimicrobial efficacy of the fractions, aliquot of test culture (100 μL) was evenly spread over the surface of the solidified agar. Bacteria were cultured on Nutrient agar; while the fungal strains were cultured on Malt extract agar, and *Candida* species was cultured on Yeast malt extract agar. Solvents either aqueous or organic solvents were used as negative control for test microorganisms. All the preloaded plates with respective extract and test organism were incubated at 37 °C, for 24 hours for bacteria and at 28 °C. for 48 hours for fungi. After the incubation period, zone of inhibition was measured in millimeters. All the tests were carried out in triplicate and their means were recorded **(Ramesh and pattar, 2010).**

2.5 Minimum inhibitory concentration (MIC) assays

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of an antifungal or antibacterial that inhibits the growth of the tested microorganism. The most active extracts were investigated to determine its MIC against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus flavus)* as drug resistant microorganisms (**Tsiodras *et al.*; 2001& Adam *et al.*; 2008)** . Different dilutions (10 - 100 ug/ml) were examined by agar diffusion method , a

control was also served; 20 µl from each of the test organisms was used to inoculate the tubes. The tubes were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungi **(Aditya *et al*., 2012).**

2.6 Statistical analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one-way analysis of variance, Differences were considered significant at $P< 0.05$ according to (**Ramesh and Pattar, 2010)**

3. Results and Discussion

3.1 Results

All mushroom extracts from fruiting bodies of both(*Pleurotus ostreatus* & *Agaricus bisporus)* (6 Fresh & 6 Dried) were examined. In this study were found to exhibit various degrees of antagonistic effects against the tested microorganisms. The fresh aqueous extract (FAP) of *Pleurotus osteratu*s showed highly antibacterial activity against all tested eight pathogenic bacteria Hence the diameter of inhibition zone was 22 mm of *Bacillus cereus* growth followed by *Klebsiella pneumonie* & *Bacillus polymexa* that showed the same zone of inhibition with diameter equal 21 mm.

The dried aqueous extract (DAP) of the fruiting bodies of the *Pleurotus ostreatus* was examined for their antibacterial activities against only seven bacterial species only shown in Figure (1) and Table (1). *Pseudomonas aeuroginosa* was the most sensitive microorganism to dried aqueous pleurotus (DAP) extract and inhibited by 23 mm followed by *Bacillus cereus* with inhibition zone 22 mm, then *Staphylococcus aureus* , *Bacillus subtilis cereus* and *Klebsiella pneumonie* were followed by same zone of inhibition equal 21mm.

The fresh ethanolic extract (FEP) of the fruiting bodies of the *Pleurotus ostreatus* was evaluated for their antibacterial activities against eight bacterial species but affected on seven species only.

As shown in the Table (1) and Figure (1) indicate that *Bacillus cereus* was the most susceptible to the fresh ethanolic extract of *Pleurotus ostreatus* and inhibited by 25 mm diameter of inhibition zone followed by *Pseudomonas aeuroginosa* with diameter of inhibition 22 mm, on contrast, *salmonella entitic* show high resist to the same extract.

Bacillus cereus was found to be the most susceptible bacteria (18 mm) (Table 1).On the other hand, *Escherichia coli, Klebsiella pneumonie* were not affected by the ethanolic extract of dried *Pleurotus ostreatus* (DEP) (Table 1 and Figure 1).

The fresh methanolic extract of the fruiting bodies of the *Pleurotus ostreatus* (FMP) was examined for their antibacterial activities and affected on four bacterial species only.*Bacillus polymexa* showed 17 mm diameter of inhibition zone,,While , growth of *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* were also inhibited by 13 & 11 and 10 mm respectively (Table 1).

On the other hand, other bacterial strains didn't affect by the fresh methanolic extract of *Pleurotus ostreatus* fruiting bodies.*Staphylococcus aureus* was the most sensitive to (DMP) with zone of inhibition 21mm. *Bacillus cereus* and *Bacillus polymexa* were also affected, the resulted inhibition zone had diameter equal 17 , 20 mm after 24h of inoculation respectively.On the other hand, *Klebsiella pneumoniae*, and *bacillus subtilis* were not affected by the dried methanolic extract of *Pleurotus ostreatus* (Table 1 and Figure 1).

Staphylococcus aureus and *Bacillus cereus* were sensetive to all pleurotus extracts whatever they are fresh or dried extracts.

The fresh aqueous agaricus extract (FAA) was the most active component against all bacterial species except *Bacillus cereus*. Shown in (Table 1 and Figure 2).

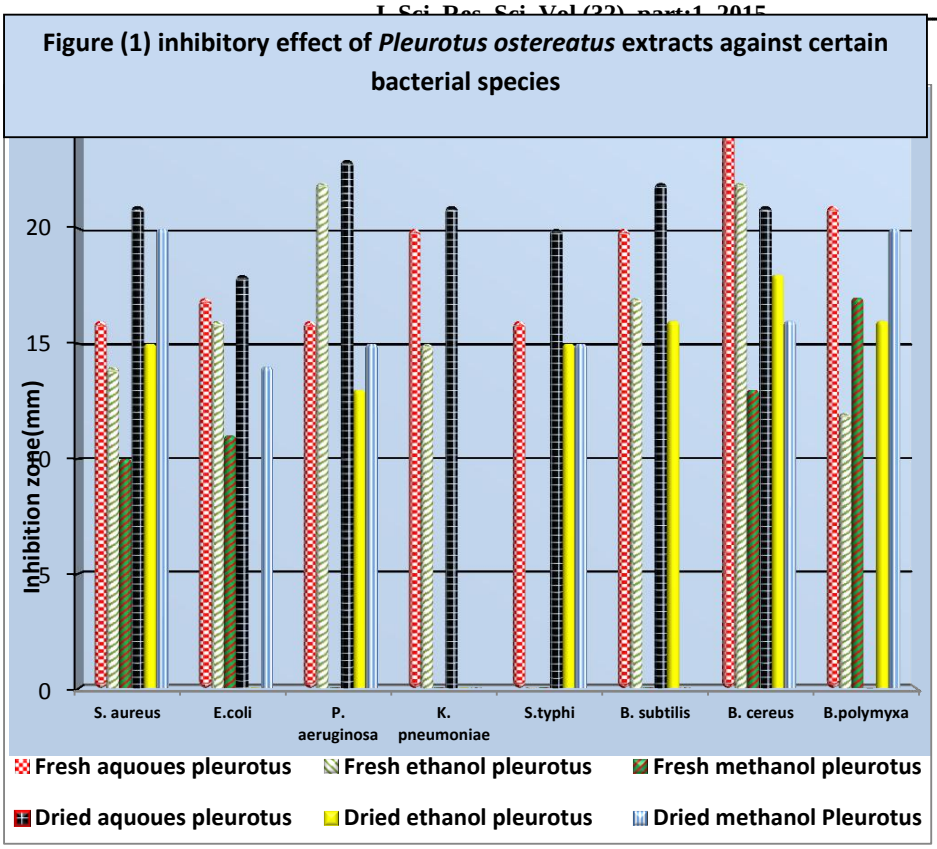
Klebsiella pneumonia was more susceptible to the (FAA) extract and inhibited by 22 mm diameter of inhibition zone, Also *Bacillus polymexa* and *Pseudomonas aeuroginosa* with same diameter of zone inhibition equal 21 mm.

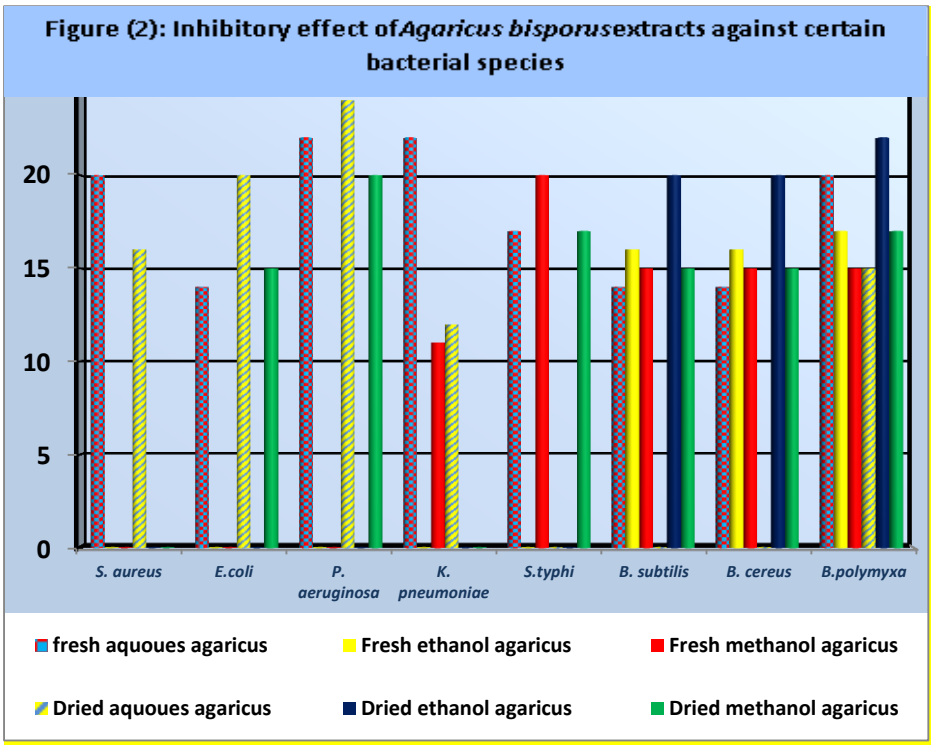
On the other hand, *Pseudomonas aeuroginosa* was the most sensitive to (DAA) and inhibited by 24 mm inhibition zone followed by *Escherichia coli* with inhibition zone equal 21mm.Bacterial growth of *Bacillus polymexa, Staphylococcus aureus* , and *Klebsiella pneumonia* was slightly affected (Table 1 and Figure 2).

Fresh and dried ethanolic agaricus extracts (FEA &DEA) have the lowest effect on the most of bacterial growth , except *Bacillus polymexa* and *Bacillus subtilis* While fresh and dried methanolic extracts (FMA &DMA) were moderate affected on *Escherichia coli,Bacillus subtilis* and *Klebsiella pneumonia* . But also both of them affected well on *Salmonella entitic, Pseudomonas aeuroginosa* and *Bacillus cereus* with inhibition zone 20, 20, 18 mm respective

Table (1): Assessment of antimicrobial activity of aqueous ,ethanolic and methanolic extracts fresh and dried mushrooms (*Pleurotus ostreatus* & *Agaricus bisporus*) against eight pathogenic bacterial strains

Mushroom	Extracts		Inhibition zone by(mm) Bacterial strains								
			Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*klebsiella*	*Salmonella enteric*	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus polymexa*	LSD at 5%
Pleurotus ostereatus	Fresh	Aqueous	16	17	16	21	17	20	22	21	1.05
		ethanolic	15	16	22	15	0.0	17	25	12	1.28
		Methanolic	10	11	0.0	0.0	0.0	0.0	13	17	1.67
	Dried	Aqueous	21	18	23	21	20	21	22	0.0	2.14
		ethanolic	15	0.0	13	0.0	15	16	18	16	1.45
		Methanolic	21	14	15	0.0	15	0.0	17	20	1.32
Agaricus bisporus	Fresh	Aqueous	20	15	21	22	17	15	0.0	21	1.58
		ethanolic	0.0	0.0	0.0	0.0	0.0	16	0.0	18	0.76
		Methanolic	0.0	0.0	0.0	12	20	15	15	15	2.16
	Dried	Aqueous	16	21	24	12	0.0	0.0	0.0	15	1.44
		ethanolic	0.0	0.0	0.0	0.0	0.0	20	19	21	0.48
		Methanolic	0.0	16	20	0.0	18	15	18	17	1.22
	LSD atT 5%		0.84	1.17	1.17	0.67	0.67	1.33	1.33	1.17	





All the fungal species were inhibited by the fresh aqueous extract from *Pleurotus ostreatus* (FAP) ,which was the most potent extract that showed high inhibition effect on the growth of

the most tested fungi. *Aspergillus flavus* was found to be the most susceptible fungi, the diameter of inhibition zone was 21 mm followed by *Aspergillus niger, Rhizopus nigricans* and *canida albicans* showing 20, 19, 18 mm inhibition zone, respectively (Table 2). On the other hand, the diameter of inhibition zone of *Penicillium expansum* and *Fusarium moniliforme* growth were reduced to 15, 16 mm respectively. On contrast, dried aqueous extract (DAP) did not inhibit any fungal growth as shown in Table (2) & Figure (3).

Aspergillus flavus was highly inhibited by fresh ethanolic extract of *Pleurotus ostreatus* (FEP) followed by *Fusarium moniliforme* and *Penicillium expansum* with zone of inhibition equal 19 & 15 & 15 respectively, while others were not affected.

However, dried methanolic extract (DEP) affect on all fungal strains, the zone of inhibition ranged from 15 mm to 19 mm (Table 2). The fresh and dried methanolic extracts (FMP &DMP) affected on *Aspergillus flavus*, *Aspergillus niger* and also on *Fusarium moniliforme,* But DMP only had slight effect on *Candida albicans* with zone of inhibition equal 12 mm.

Rhizopus , Candida albican fungal growth affected only by (FAP & DEP) but also *Candida albican* affected by DMP.The most susceptible fungal growth to pleurotus fresh and dried extracts were *Aspergillus flavus*, *Aspergillus niger* with inhibition zone ranged from 15 mm to 21mm .(Table 2 & figure 4)

The fresh aqueous extract of the fruiting bodies of *Agaricus bisporus* (FAA) was the most potent extract that showed high inhibition effect on the growth of the most tested fungi.It exerted 22 mm inhibition zones with diameter equal 22 mm against *Aspergillus niger,* and 20 mm for *Aspergillus flavus* & *Rhizopus nigrecans* .Also, *Candida albican*s and *Fusarium moniliforme* were highly susceptible to this extract, the diameter of inhibition zones were 18 mm ,15 respectively.While, (DAA) inhibited only the growth of *Aspergillus flavus* and *Penicillium expansum* with moderate and slight inhibition zone (Table 2& Figure 5) .Both FEA &DEA affected on the growth *Fusarium moniliforme* with inhibition zone 19 mm and 15 respectively.Also, *Aspergillus niger* and *Aspergillus flavus* were highly inhibited by the fresh ethanolic extract of *Agaricus.*and *Penicillium expansum* was inhibited by DEA but with slight effect.On the other hand, the fresh and dried methanolic extracts were affected on all fungal species except *Rhizopus* which was resistant to (FMA). *Aspergillus niger* was the most susceptible to (FMA) with zone of inhibition equal 21 mm

Table (2): Assessment of antimicrobial activity of aqueous ,ethanolic and methanolic extracts fresh and dried mushrooms (*Pleurotus ostreatus* & *Agaricus bisporus)* against six pathogenic fungal strains

Mushroom		Extracts	Inhibition zone by(mm)						
			Fungal Strains						
			Aspergillus flavus	*Aspergillus niger*	*Penicillium expansum*	*Fusarium moniliforme*	*Rhizopus*	*Candida albicans*	LSD at 5%
Pleurotus ostereatus	Fresh	Aquoes	21	20	15	16	19	18	0.89
		Ethanolic	19	0.0	15	15	0.0	0.0	0..68
		Methanolic	15	16	0.0	19	0.0	0.0	0.92
	Dried	Aquoes	0.0	0.0	0.0	0.0	0.0	0.0	NS
		Ethanolic	17	19	19	18	15	18	0.78
		Methanolic	15	16	0.0	20	0.0	12	1.18
Agaricus bisporus	Fresh	Aquoes	20	22	0.0	15	20	18	1.28
		Ethanolic	16	17	0.0	19	0.0	0.0	0.98
		Methanolic	15	21	15	16	0.0	13	1.36
	Dried	Aquoes	17	0.0	12	0.0	0.0	0.0	1.45
		Ethanolic	0.0	0.0	14	15	0.0	0.0	0.91
		Methanolic	15	19	17	14	17	11	1.22
	LSD atT 5%		1.17	0.83	1.00	1.17	0.50	1.17	

Figure (3) inhibitory effect of *Pleurotus ostereatus* extracts against certain bacterial species

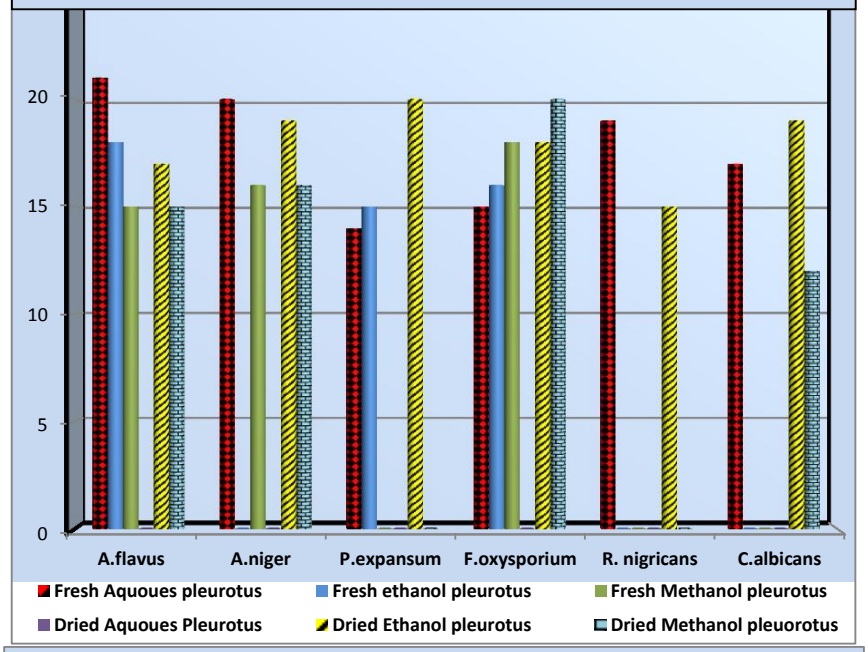
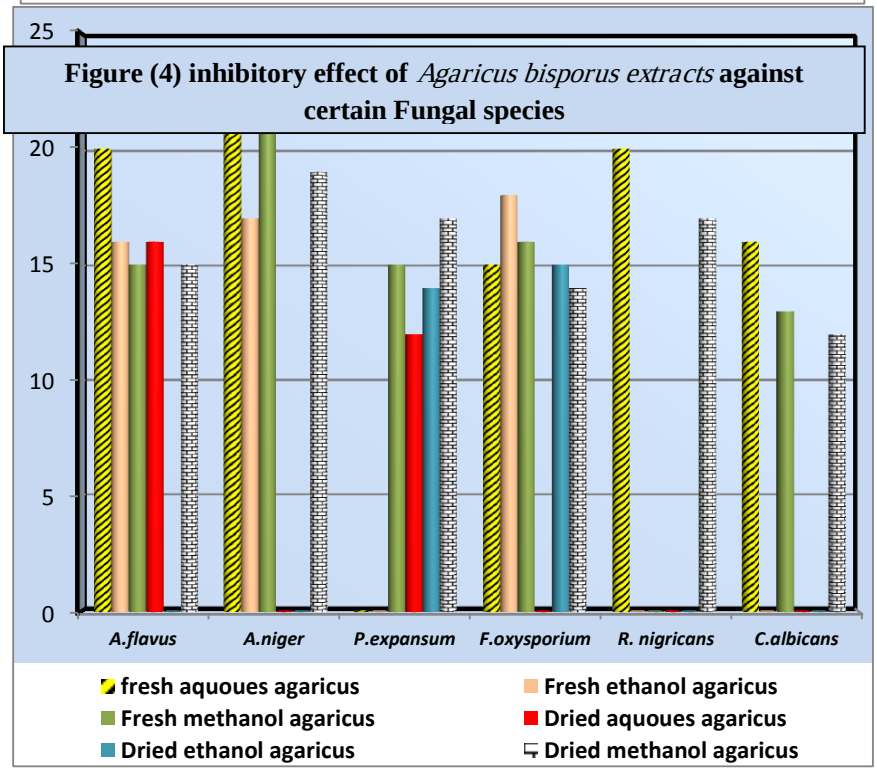


Figure (4) inhibitory effect of *Agaricus bisporus extracts* against certain Fungal species



The result in Table (3) indicated that the MIC of FAP was 30, 20 µg/ml in case of bacterial strains; *Staphylococcus aureus* and *Escherichia coli* respectively. While, the MIC was 30 µg/ml in case of the two fungal strains; *Candida albicans* and *Aspergillus flavus*

Furthermore, the MIC of DAP was 30, 40 µg/ml for both *Staphylococcus aureus* and *Escherichia coli* respectively.Also, the MIC of FAA was 20 µg/ml for gram positive (*Staphylococcus aureus)* and gram negative (*Escherichia coli*) ; 30 µg/ml for the yeast (*Candida albicans*) and 40 µg/ml for the filamentous fungi (*Aspergillus flavus)*.While, the MIC of DAA was 20, 30 µg/ml for both *Staphylococcus aureus* and *Escherichia coli* respectively and 50 µg/ml was the MIC of compound (E) in case of *Aspergillus flavus.*

Finally, FEP with MIC 30, 20 µg/ml for both *Staphylococcus aureus* and *Escherichia coli* respectively and 40 µg/ml was the MIC in case of *Aspergillus flavus.*

Table (3): MIC of the active antimicrobial extracts against certain pathogenic microorganisms.

Active Extract	**Test microorganisms**			
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***	***Aspergillus flavus***
	Concentration of active compound (µg/ml)			
FAP	**30**	**20**	**30**	**30**
DAP	**30**	**40**	**0**	**0**
FAA	**20**	**20**	**30**	**40**
DAA	**20**	**30**	**0**	**50**
FEP	**30**	**20**	**0**	**40**

3.2Discussion

In our study, the aqueous extract of *P. ostreatus (*fresh or dried) was the most potent due to its high inhibitory effect against most tested bacterial and fungal organisms.

This result is in accordance with those reported by (**Hearst *et al.*,2009)** who isolated antimicrobial compound from the aqueous extract of both *Lentinula edodes* and *Pleurotus ostreatus* fruiting bodies against certain bacterial and fungal pathogens. The aqueous extract of fresh fruiting bodies of the *P .ostreatus* had inhibitory effect on mycelial growth of *Fusarium oxysporum, Mycosphaerella arachidicola* and *Physalospora piricola* **(Chu *et al.*, 2005)** as well as *Aspergillus niger* **(Chu *et al.*, 2006**).This result is in accordance with those reported by (**Miguel *et al.*,1997)** who demonstrated that volatile compounds secreted by the oyster mushroom (*Pleurotus ostreatus*) have strong antibacterial activity.

Also, **(Youssef *et al.*,2008)** reported the production of cellulase complex enzymes from aqueous extract of *Pleurotus ostreatus* which showed strong inhibition activity against different bacterial strains including *Mycobacterium aurum, Staphylococcus aureus, Streptococcus* sp., *Acinetobacter calcoaceticus* and *Klebsiella* sp.

Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae,Bacillus cereus and *Pseudomonas aeruginosa* also were highly susceptible to Aqueous extract of *P.ostereatus* which showed 30 mm, 29 mm, 27 mm, and 22 mm diameter of inhibition zone of bacterial growth, respectively.This result was in agreement with those obtained by(**Gezer *et al.*,2006)** who demonstrated that the water extract from edible mushroom *Ramaria flava* inhibited the growth of Gram positive and negative bacteria.

On the other hand aqueous extracts from *A. bisporus* gave a highly effect on bacterial and fungal species ,Antimicrobial activity of *A. bisporus* must have been due to the presence of essential bioactive components (***Abah and G. Abah* ,2010) .**The susceptibility of these organisms to *A. bisporus* methanolic extract denotes the use of these macrofungus in the treatment of infections with these organisms as aetiologic agents. Addition of these macrofungus as additives to food could reduce occurrences of infections due to foodborne pathogens such as *Bacillus cereus.* It has been documented that certain extractable product from macrofungus can be used as supplements in human diet to enhance health and fitness because of their medicinal properties (***Abah and G. Abah* ,2010).**

(**Mohammad *et al*., 2012)** stated that various kinds of proteins with several biological activities are produced by mushrooms. In order to evaluate antibacterial activity of edible mushrooms, we isolated proteins of *Agaricus bisporus* and examined their effects on gr + and gr- bacteria. Antibacterial activity of total extract proteins as well as protein fractions was evaluated by the method of micro dilution against gr+ and gr- bacteria. The isolated proteins

from the mushroom, *Agaricus bisporus* fruiting bodies were effective against *Staphylococcus aureus*. The proteins of edible mushrooms like *Agaricus bisporus*, maybe viewed as a natural source of antibacterial agents.**)**

β-glucans from yeast and mushrooms and pectin have anti-infectious properties in rodent models against different microbes, including mycobacteria.. An *Agaricus* extract, has been shown to protect against both Gram-positive and Gram -negative sepsis in mice and has been tested against viral infections, as reviewed here. Thus, in the future, biologically active substances isolated from medicinal mushrooms and plants, may prove useful alternatives in the fight against serious infections **(Hetland *et al*., 2013).**

The effective factor in the extracts of mushrooms that have antimicrobial properties is considered to be disaccharides sugar, Trehalose. *Pleurotus ostereatus* and Agaricus bisporus extracts can improve the beneficial intestinal flora of the gut and reduced harmful effects of certain bacterial enzymes such as β-glucosidase , β- glucorinidase and tryptophase as well as reducing colon cancer **(Mahendra *et al* .,2005**).

4. Conclusion

In conclusion, the results showed the antimicrobial importance of *Pleurotus ostereatus* and *Agaricus bisporus* tested, and they are commonly consumed as edible mushrooms in Egypt as well as in the whole world with their delicious taste.

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الملخص العربي

دراسة معملية علي النشاط المضاد للميكروبات لنوعين من عيش الغراب المأكول (*الأجاريكس بايسبورس*) و(*البلوروتس اوستراتس*)

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تم اجراءالدراسة الحالية معمليا لتقييم النشاط المضاد للميكروبات لنوعين من عيش الغراب (*البلوروتس اوستراتس والأجاركس بايسبورس*) في الحالة الطازجة او المجففة باستخدام المستخلصات المائية او العضوية . وقد تم اجرء الدراسة علي مجموعة من الكائنات الممرضة من البكتريا والفطريات .وباستخدام طريقة الفجوات المنتشرة علي الاجار وقد أوضحت النتائج ان البكتريا الموجبة لصبغة جرام اكثرهم تأثرا وخصوصا النوعين *باسيلاس بوليمكسا وستاف اوريس* بينما الفطريات *اسبرجيلس فلافس وفيوزاريم* اكثر حساسية لكل من المستخلصات.وجد ايضا أن المستخلص المائى للاجسام الثمرية لفطر *البليروتس أوستراتس* كان له الأثر الفعال في تثبيط الكائنات الممرضة اكثر من *الاجاركس بايسبورس*.وقد لوحظ ان الحد الادني للتركيز المثبط تراوح بين (٢٠- ٤٠ ميكروجرام/مل) للبكتريا و(٣٠-٥٠ ميكروجرام/مل ميكروجرام/مل) للفطريات وكذلك يتضح ان التناول اليومي لعيش الغراب قد يمد الجسم بمضادات حيوية طبيعية لمقاومة الكائنات الممرضة