

Evaluation of Antimicrobial Activities of Mycelia and Crude Extracts of some Egyptian Wild Mushrooms, *Agaricus* and *Ganoderma* Species

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ABSTRACT

The current study focuses on the evaluation of the antimicrobial activities of some Egyptian wild mushrooms: two strains of *Agaricus* namely; *Agaricus bitorquis* (A40), *Agaricus bisporus* (A60), and three species of *Ganoderma* (G1, G3, G7). They were investigated against 13 pathogenic microorganisms (eight fungal spp and five bacterial spp). The mycelia of A40, G7, and G3 were the most effective on the largest number of pathogenic microorganisms. Antimicrobial activities of the crude extracts of (A40, G7, G3) by different solvents (methanol, water & ethylacetate) were examined by the agar well diffusion method. The results revealed that these mushrooms have potential activities as natural antifungal and antibacterial. The highest antagonistic effect of mushroom extracts was exhibited by ethylacetate extract of *Ganoderma* (G7) against *F. oxysporum* and *Ganoderma* (G3) is the most effective against *Salmonella typhi*. The mushroom extracts have lower antifungal and antibacterial activities comparing with antifungal except ethylacetate extracts of both *Ganoderma* (G3) and *Agaricus bitorquis* (A40) exhibited higher inhibition effect against *Curvularia sp.*

INTRODUCTION

Mushrooms are macrofungi that have distinctive fruiting bodies which can be either epigeous or hypogeous, large enough to be seen by naked eye and to be picked by hand (Chang and Miles, 1992). Chinese and Egyptians were among the first peoples to appreciate the medicinal value of mushrooms. Egyptians associated mushrooms with immortality and since they revered their Pharaohs, they included mushrooms as a specialty in the diet of the royal family. Mushrooms have been investigated as functional foods and as a source for the development of nutraceuticals and medicines (Lindequist *et al.*, 2005; Poucheret *et al.*, 2006). Infectious diseases remain one of the major threats to human health (Yamac and Bilgili, 2006). The development of antibiotics has been one of the most significant scientific achievements of the last seventy years. These compounds act in several ways, by interfering in the structures and metabolic processes of organisms (Fuchs, 2004). Recently, it is reported that bacteria develop resistance to antibiotics through genome mutations that are crucial for their survival (Munita and Arias, 2016). Although numerous antibiotics have been used against pathogens, antimicrobial resistance is an increasing public health problem (Yamac and Bilgili, 2006 and WHO, 2018).

Natural resources have been discovered in the last years and among them mushrooms as an alternative source of new antimicrobials. Mushrooms need antimicrobial compounds to struggle against the competitors and survive in their natural environments. Therefore, antimicrobial compounds with less or more strong activities could be isolated from many mushrooms and they could be benefit for human (Lindequist *et al.*, 1990; Ponugupati, 2015 and Shen *et al.*, 2017).

Mushrooms are rich sources of natural antibiotics as their cell wall glucans are recognized by their immunomodulatory properties and their externalized secondary metabolites that combat viruses and bacteria (Brandt and Piraino, 2000 and Akyuz *et al.*, 2010). Fruiting bodies, mycelia, and spores accumulate different kinds of bioactive metabolites with immunomodulatory, antioxidant, anti-inflammatory, antitumor and antimicrobial properties (Goncalves *et al.*, 2011). Most of the medicinal mushrooms extracts contained different forms of polysaccharides that strengthen the immune system, lysozyme, bacteriolytic

enzyme, and acid protease (Klaus & Niksic, 2007). *Ganoderma* species were investigated to have triterpenoids or polysaccharides and used against more often in combination with chemotherapeutic agents which have been used to treat various bacterial diseases and against Hepatitis B virus, Herpes simplex virus, Epstein-Barr virus, and human immunodeficiency virus (HIV) in vitro or in animal models (Smania *et al.*, 2007; Qureshi *et al.*, 2010 and Benkeblia, 2015). The objective of the present study is to high light the importance of wild mushrooms and its antimicrobial activity against pathogenic bacteria and fungi.

MATERIALS AND METHODS

Collection of fungal fruiting bodies

Fruiting bodies of *Ganoderma spp* (G1,G3,G7) were collected from some trees at Mansoura University Campus, Dakhliya governorate_ Egypt and from citrus farm at El-Sinania, Damietta governorate_Egypt. *Agaricus bisporus* (A60) and *Agaricus bitorquis* (A40) were collected from National park of New Damietta. All of these mushrooms had been identified and isolated previously at Botany and Microbiology Department, Faculty of Science, Damietta University.

Tested organisms

The stored cultures of *Escherichia coli*, *Salmonella typhi*, *Erwinia carotovora*, *Staphylococcus aureus* and *Streptococcus sp.* were supplied from Bacterial Laboratory of Faculty of Science and Microbiological Laboratory of Faculty of Agriculture, Mansoura University. Strains of pathogenic fungi *Aspergillus flavus*, *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Penicillium italicum*, *Penicillium purpurogenum*, *Curvularia sp.* and *Penicillium citrinum* were supplied from the Fungal Laboratory, Botany Department., Faculty of Science, Mansoura University.

Antimicrobial activity of mushrooms mycelia by Agar disc method

0.1ml of the tested microbial suspension (10^{10} CFU/ml) was transferred using sterile pipette to the center of the potato dextrose agar (PDA) plate for fungi and nutrient agar for bacteria and spread by sterile glass spreader separately. Then 4mm diameter of each mushroom mycelial disc was cut using a sterile cork borer then placed in the center of the above plates separately under aseptic condition. The cultures were incubated at

room temperature in dark for 3-5 days for the appearance of clear zones around the disc. The indication of inhibition was therefore observed (SM et al., 2009). The experiments were conducted in 3 replicates.

Preparation of crude mushrooms extract

Fresh fruiting bodies were dried in shade conditions and 40 grams of the dried material was pulverized in the blender to get a coarse powder then soaked in Erlenmeyer flask separately in 300ml of water, ethyl acetate, and methanol for water, ethyl acetate, and methanol extracts. The flasks were covered with foil then allowed to stand for 8 days for extractions. These extracts were filtered through filter paper and concentrated using rotary evaporator under reduced pressure at 40°C. All the extracts were stored in air tight containers in a refrigerator. The extracts were analyzed for antifungal and antibacterial activity (Balakumar et al., 2011).

Well diffusion method

Antimicrobial activity (antibacterial and antifungal activity) of different mushrooms extracts using well diffusion method was tested (Bauer et al., 1996). The plates of the prepared cultures were inoculated with different fungi and bacteria. Wells with (six mm) cork borer were made on the agar surface. The 100 microliters of different mushroom extracts were poured into the wells

using sterile syringe. Then the plates were incubated for 3-4 days at 28°C for fungal activity and for 24 hours at 35±2 °C for bacterial activity. The plates were observed for the inhibition zone formation around the wells. The inhibition zone was calculated by measuring the diameter of the well and the diameter of the inhibition zone around the well. Inhibitory activity of DMSO was also tested. Nystatin (antifungal) and pencillin (antibacterial) were used as controls. The readings were taken in 3 different fixed directions in all three replicates and the average values were tabulated and compared with the control.

RESULTS AND DISCUSSION

Mushrooms are rich sources of natural antibiotics as their cell wall glucans are recognized by their immunomodulatory properties and their externalized secondary metabolites that combat viruses and bacteria (Brand and Piraino, 2000 and Akyuz et al., 2010).

The effect of mycelia of *Ganoderma* and *Agaricus* species on the growth of pathogenic microorganisms was presented in table 1. The data were analyzed using SPSS 24 for Windows. Friedman Test used for comparing the difference between the effect of (G1,G3,G7,A40,A60) on pathogenic microorganisms.

Table 1. Effect of G1, G3, G7, A40, and A60 on the tested pathogenic microorganisms Inhibition zone represented in (mm):

Tested organism	G1	G3	G7	A40	A60	x ²	p
	μ ± std	μ ± std	μ ± std	μ ± std	μ ± std		
<i>Fusarium solani</i>	0.00	21.03	20.0	0.00	0.00	12	0.017<0.05
	0.00	0.45	0.20	0.00	0.00		
<i>Curvularia sp.</i>	33.30	4.99	0.00	6.06	0.00	12	0.017< 0.05
	0.10	0.20	0.00	0.15	0.00		
<i>Rhizoctonia solani</i>	0.00	5.06	0.00	7.00	0.00	12	0.017< 0.05
	0.00	0.95	0.00	0.10	0.00		
<i>Fusarium oxysporum</i>	0.00	0.00	19.06	16.03	27.03	12	0.017< 0.05
	0.00	0.00	0.49	0.35	0.20		
<i>Penicillium italicum</i>	0.00	0.00	26.0	14.03	0.00	12	0.017< 0.05
	0.00	0.00	0.26	0.05	0.00		
<i>Penicillium citrinum</i>	37.00	0.00	38.03	0.00	0.00	12	0.017< 0.05
	0.20	0.00	0.55	0.00	0.00		
<i>Aspergillus flavus</i>	0.00	0.00	0.00	5.06	0.00	12	0.017< 0.05
	0.00	0.00	0.00	0.90	0.00		
<i>Penicillium purpurogenum</i>	25.93	0.00	0.00	0.00	0.00	12	0.017< 0.05
	0.05	0.00	0.00	0.00	0.00		
<i>E.coli</i>	18.00	36.06	13.00	9.06	7.06	12	0.02< 0.05
	0.26	0.52	0.30	0.95	0.11		
<i>Staphylococcus aureus</i>	18.01	20.06	10.03	5.00	0.00	12	0.017<0.05
	0.26	0.15	0.25	0.40	0.00		
<i>Salmonella typhi</i>	19.00	19.03	0.00	0.00	17.03	11.439	0.017< 0.05
	0.20	0.45	0.00	0.00	0.25		
<i>Erwinia carotovora</i>	0.00	0.00	10.03	11.03	4.00	12	0.017< 0.05
	0.00	0.00	0.55	0.25	0.40		
<i>Streptococcus sp.</i>	0.00	0.00	0.00	4.06	0.00	12	0.017< 0.05
	0.00	0.00	0.00	0.49	0.00		

Antimicrobial activity of mushroom mycelia (G1, G3, G7, A40, A60) were tested against 13 pathogenic microorganisms. All the mushrooms used in this study were found to exhibit various degrees of antagonistic effects against the tested pathogenic microorganisms. This was evidenced by the clear inhibition zone of bacteria and fungi around the tested mushroom mycelia. It is clear from table 1 that A40 was the most efficient fungus against

almost the tested pathogenic microorganisms (9 from 13). It shows higher antibacterial (4 from 5) than antifungal activity (5 from 8). In general, all the listed mushrooms show higher antibacterial activity than antifungal activity. The least efficient antifungal mushroom was A60 which showed inhibitory effect against *Fusarium oxysporum* only. All the tested pathogenic fungi are inhibited by the mushrooms mycelia in different degrees. The most difficult

to be inhibited were *Penicillium purpurogenum* and *Aspergillus flavus* which were inhibited by only one mushroom mycelium; G1 & A40 respectively, followed by *Penicillium citrinum*, *Fusarium solani*, *Penicillium italicum*, and *Rhizoctonia solani* which were inhibited by two mushrooms, G1 & G7 for the former, G7 & G3 for the second, A40 & G7 against the third and G3 & A40 against the fourth. The highest inhibition activity of A60 & A40 (27.03mm, 16.03mm) were against *Fusarium oxysporum*; while G7 & G1 (38.03mm, 37.0mm) were against *Penicillium citrinum* and G3 caused its highest inhibition effect on *Fusarium solani* (21.03mm). The inhibition of *Curvularia sp* was observed to be high (33.3mm) by G1 and moderate by both A40 (6.06mm) and G3 (4.9mm).

On the other hand, G7 & A40 did not show any activity against *Salmonella typhi*, while the G1, G3, A60 did (19.0, 19.03 & 17.03 mm inhibition zones respectively). *Ganoderma spp* inhibited the growth of *Staphylococcus aureus*; G3 showed the maximum activity (20.06mm) as seen table 1, but the inhibition was moderate by G1 (18mm) & G7 (10mm). Although A60 did not show any activity against *Staphylococcus aureus*, G1, G3 & G7 showed high effect and recorded inhibition zone (18.0, 20.06 & 10.03mm), respectively while, A40 had the lowest inhibitory (5mm). It was observed that G3 showed high activity against *E.coli* (36mm), moderate by G1 (18.0mm) and G7 (13.0mm) but, low in case of A40 (9.06mm) and A60 (7.06mm). Mycelia of G1 & G3 did not show any effect against *Erwinia carotovora*, but its growth was showed to be reduced by G7 (10.03mm), A40 (11.03mm), and A60 (4.0mm). G1, G3, G7, A60 did not show any effect against *Streptococcus sp.* but the inhibition activity was showed by A40 only (4.06mm). In table 1, it was observed that G1 has activity against *Penicillium citrinum* recorded inhibition zone (37.0mm), *Penicillium purpurogenum* (25.9mm), *Curvularia sp.* (33.3mm), *Salmonella typhi* (19.0mm), *Staphylococcus aureus* (18.0mm) and *E.coli* (18.0mm). G3 has inhibitory effect on *Curvularia sp.* (4.9mm), *Fusarium solani* (21.03mm), *Rhizoctonia solani* (5.06mm), *Staphylococcus aureus* (20.06mm), *Salmonella typhi* (19.03mm), *E. coli* (36.06mm) only. A60 has the highest activity against *Fusarium oxysporum* (27.03mm), moderate against *Salmonella typhi* (17.03mm), weak against *E. coli* (7.06mm) and *Erwinia carotovora* (4.0mm). G7 has the highest activity against *Penicillium citrinum* (38.03mm), and *Penicillium italicum* (26.0mm), moderate against *Fusarium solani* (20.0mm), *Fusarium oxysporum* (19.06mm), *E.coli* (13.0mm), *Staphylococcus aureus* (10.03mm) and *Erwinia carotovora* (10.03mm). A40 has the highest activity against *Fusarium oxysporum* (16.03mm), *Penicillium italicum* (14.03mm), moderate against *Erwinia carotovora* (11.03mm), *E.coli* (9.06mm), *Rhizoctonia solani* (7.0mm), weak against *Streptococcus sp.* (4.06mm), *Staphylococcus aureus* (5.0mm), *Curvularia sp.* (6.06mm), and *Aspergillus flavus* (5.06mm).

The p value for is significant, $p > 0.05$, that's means there's statistically significant difference between (G1,G3,G7,A40,A60) on pathogenic microorganisms. *Agaricus bitorqus* A40, *Ganoderma* 3 & 7 were chosen for further study because they were more efficient as antimicrobial agents.

Antibiosis is a biological interaction between two or more organisms that is detrimental to at least one of them; it can also be an antagonistic association between an organism and the metabolic substances produced by another. Ofodile and Bikomo (2008) reported antimicrobial screening of various solvent extracts of *Ganoderma lucidum* on some bacterial species, some bioactive components such as alkaloids, terpenoids, and phenolics were investigated which could also be the reason of the activity of mycelial culture of *Ganoderma lucidum*. The present results proved that mushrooms mycelia and different solvent extracts of mushroom fruiting bodies have antimicrobial effects. Other researchers reported the changeable of antimicrobial activity of *A. bisporus*, *Pleurotus spp.*, and *T. boudieri* (Uzun *et al.*, 2004; Demirhan *et al.*, 2007; Iwalokun *et al.*, 2007 and Jagadish *et al.*, 2008), which may arise from the genetic structures of mushroom species, biochemical, physical constituents, chemical differences of mushroom solvents, extracts and tested microorganisms that other research shows clearly when it is compared to the other mushroom species (Wang *et al.*, 2004; Gao., *et al* 2005 and Lindequist *et al.*, 2005). They claimed that the sensitivity of microorganisms to chemotherapeutic compounds change even against different strains.

Species of mushrooms have various constituents and in different concentration, which account for the differential antimicrobial effect, as reported. The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolites of different chemical types in mushroom compounds. Suay *et al.* (2000) observed the intra-specific genetic differences of mushroom species. They reported different antibiotic activity in mycelial cultures of 204 mushroom species. This observation was in agreement with the present results. They also observed the antimicrobial activity of the extracts of mycelial cultures of several *Ganoderma* species, as our results revealed for the three species of *Ganoderma*. According to Chang, (2001), products of mycelium are the "wave of the future" as they ensure year-round production and ensure standardized quality. Previous reports investigated that various strains from the same species produced different amounts of antimicrobial compounds (Sidorova and Velikanov, 2000). Similar trend was obtained for *A.bitorquis* (A40) and *A. bisporus* (A60). G7 and A40 inhibited *Erwinia carotovora* in agreement with investigation of the mycelial leachate of *L. edodes* that contained substances that make suppression to other plant pathogens, such as *Erwinia amylovora*, *P. syringaepv. Tabaci*, and *Curtobacterium flaccum faciens*pv. *Flaccum faciens* (Pacumbaba *et al.*, 1999). The results showed the efficient inhibition of the three *Ganoderma spp* to *Staphylococcus aureus*. In this instance, Coletto and Mondino (1991) reported that culture extracts and methanolic extracts of *G. recinaceum* mycelia inhibited *Staphylococcus aureus*.

The results presented in table 2 showed that the ethyl acetate extract of A40 has the positive effect against the largest number of tested organisms followed by the extract of G7. G3 extract has positive effect against the lowest number of the tested organisms. The same effect of mushroom mycelia on the tested microorganisms was

shown by ethyl acetate fruit body extract of all the three tested mushrooms. The extract of A40 has significant inhibitory effect against *P. italicum* that give inhibition

zone (15.3mm) which near to Nystatin (17.0mm) on contrast, G7 extract has a lower inhibition effect to *P. italicum* (5.03mm) than its mycelia (26.0mm).

Table 2. The antimicrobial effect of ethylacetate crude extracts of fruiting bodies of A40, G3 and G7 on the tested pathogens Inhibition zone represented by (mm):

Tested organism	Ethylacetate extracts				
		G3	G7	A40	(nystatin/pencillin)
<i>Fusarium solani</i>	Mean	10.00*	9.06	0.00	21.03
	Std. Deviation	0.10	1.00	0.00	0.06
	Lsd Sig.	0.00	0.00	-	0.001
<i>Curvularia sp.</i>	Mean	17.03*	0.00	17.00*	15.03
	Std. Deviation	0.25	0.00	0.20	1.05
	Lsd Sig.	0.00	-	0.00	0.00
<i>Rhizoctonia solani</i>	Mean	13.06*	0.00	9.06	16.00
	Std. Deviation	0.11	0.00	1.00	0.76
	Lsd Sig.	0.00	-	-	0.01
<i>Fusarium oxysporum</i>	Mean	0.00	18.03*	10.00	21.00
	Std. Deviation	0.00	0.45	1.00	0.46
	Lsd Sig.	-	0.00	-	0.02
<i>Penicillium italicum</i>	Mean	0.00	5.03	15.36*	17.00
	Std. Deviation	0.00	0.32	0.49	1.00
	Lsd Sig.	-	-	0.00	0.001
<i>Penicillium citrinum</i>	Mean	0.00	12.06*	0.00	18.53
	Std. Deviation	0.00	1.00	0.00	0.55
	Lsd Sig.	-	0.00	-	0.00
<i>Aspergillus flavus</i>	Mean	0.00	0.00	4.03*	12.07
	Std. Deviation	0.00	0.00	0.55	0.90
	Lsd Sig.	-	-	0.00	0.001
<i>E.coli</i>	Mean	10.00*	5.03	4.07	20.50
	Std. Deviation	0.20	1.05	1.00	1.04
	Lsd Sig.	0.00	-	-	0.00
<i>Staph. aureus</i>	Mean	12.06*	10.33	6.03	21.81
	Std. Deviation	0.49	0.45	0.25	0.15
	Lsd Sig.	0.00	-	-	0.00
<i>Salmonella typhi</i>	Mean	15.00*	0.00	0.00	25.06
	Std. Deviation	0.40	0.00	0.00	0.95
	Lsd Sig.	0.00	-	-	0.00
<i>Erwinia carotovora</i>	Mean	0.00	9.30*	6.00	17.30
	Std. Deviation	0.00	0.40	0.20	0.26
	Lsd Sig.	-	0.00	-	0.00
<i>Streptococcus sp.</i>	Mean	0.00	0.00	8.00*	20.00
	Std. Deviation	0.00	0.00	0.26	0.30
	Lsd Sig.	-	-	0.00	

*. The mean difference is significant at the $p < 0.05$ level. /POSTHOC=LSD ALPHA(0.05).

A40 and G3 ethylacetate extracts have the same significant effect for inhibiting *Curvularia sp.* (17.0mm) which were more than Nystatin (15.03mm) and in comparison with their mycelia they achieved three and four times more. A40 effect on *F. oxysporum* inhibition was lower (10.0mm) than its mycelial effect (16.03mm) and half the effect of Nystatin (21.0mm) while, G7 has significant inhibitory effect recording inhibition zone (18.03mm) which was near to its mycelial effect (19.06mm). The inhibition of *R. solani* by A40 was about half (9.06mm) the inhibition of Nystatin (16.0mm) but the effect of G3 ethyl acetate extract (13.06mm) was near to Nystatin and two and half fold its mycelial effect (5.06mm). As shown in table 1 and 2 A40 is the only mushroom that has an inhibition effect on *A. flavus*. It was found also that G3 and G7 have the significant effect on *F. solani* (10.0, 9.06mm) which was half the effect of Nystatin and their mycelial effect.

G3 ethylacetate extract was significantly effective against *E.coli* that give inhibition zone (10.0mm) whereas, G7 and A40 ethylacetate extracts were not significantly effective against *E.coli* and recorded inhibition zones 5.03 & 4.06mm respectively. Ethylacetate extracts of G3 was significantly effective against *Staphylococcus aureus* that give inhibition zone (12.06mm) followed by G7 ethylacetate extract that give inhibition zone (10.3mm) while A40 has the lowest inhibition effect (6.03mm) on *Staphylococcus aureus*. *Salmonella typhi* was only inhibited by G3 which give inhibition zone (15.0mm). Ethylacetate extract of G7 was significantly effective against *Erwinia carotovora* that give inhibition zone (9.3mm) followed by A40 extract recorded (6.0mm), but G3 extract hasn't any effect. Ethylacetate extract of A40 was the only effective against *Streptococcus sp.* (8.0mm).

The results presented in table 3 showed the effect of methanol fruiting bodies crude extracts of mushrooms against the tested pathogenic bacteria and fungi. The methanolic extract of G7 was significantly effective against *Fusarium solani* that give inhibition zone (10.3mm) followed by G3 methanolic extract recorded inhibition zone (9.5mm), but A40 extract did not show any effect against *Fusarium solani*. Methanolic extracts of both G3 and A40 have the same effect against *Curvularia sp* that give inhibition zones (15.0mm), but G7 did not show activity against *Curvularia sp*. The methanolic extract of G3 was significantly effective against *Rhizoctonia solani* recorded inhibition zone (12.0mm) followed by A40 (9.03mm), but G7 hasn't any effect. Methanolic extract of A40 was significantly effective against each of *Fusarium oxysporum* (12.0mm) & *Penicillium italicum* (15.6mm) followed by that of G7 (7.06, 9.0mm respectively), while G3 did not show any effect. G7 methanolic extract was significantly effective against *Penicillium citrinum* and recorded inhibition zone (9.3mm), which was lower than

the effect of ethylacetate extract. The same effect of A40 mycelia, ethylacetate and methanolic extracts was noticed against *Aspergillus flavus* that give inhibition zone (5.066, 4.03 & 5.03mm respectively). On the other hand, G3 methanolic extract was significantly effective against *E.coli* and recorded inhibition zone (14.06mm) whereas, G7 and A40 recorded non-significant effect against *E.coli* and give inhibition zones 7.06 & 6.03mm, respectively. Methanolic extracts of G3 was significantly effective against *Staphylococcus aureus* that give inhibition zone (12.2mm) followed by G7 methanolic extract that give inhibition zone (9.3mm) and A40 methanolic extract that give inhibition zone (8.0mm). Methanolic extract of A40 was significantly effective against *Erwinia carotovora* that give inhibition zone (8.0mm) followed by G7 with inhibition zone (7.3mm), but G3 extract did not show any effect against *Erwinia carotovora*. The result revealed also that, G3 extract only was significantly effective against *Salmonella typhi*, while A40 extract only in case of *Streptococcus sp.* as shown in table 2 & 3.

Table 3. The antimicrobial effect of methanolic crude extracts fruiting bodies of A ,40G 3and G 7against tested pathogens .Inhibition zone represented by (mm):

Tested organism		Methanol extracts			
		G3	G7	A40	(nystatin/pencillin)
<i>Fusarium solani</i>	Mean	9.50	10.33*	0.00	21.03
	Std. Deviation	0.36	0.55	0.00	0.06
	Lsd Sig.	-	0.36	-	0.001
<i>Curvularia sp.</i>	Mean	15.00*	0.00	15.00*	15.03
	Std. Deviation	0.10	0.00	0.40	1.05
	Lsd Sig.	0.00	-	0.00	0.00
<i>Rhizoctonia solani</i>	Mean	12.00*	0.00	9.03	16.00
	Std. Deviation	0.46	0.00	0.06	0.76
	Lsd Sig.	0.00	-	-	0.01
<i>Fusarium oxysporum</i>	Mean	0.00	7.06	12.00*	21.00
	Std. Deviation	0.00	0.11	0.20	0.45
	Lsd Sig.	-	-	0.00	0.02
<i>Penicillium italicum</i>	Mean	0.00	9.00	15.63*	17.00
	Std. Deviation	0.00	0.20	0.25	1.00
	Lsd Sig.	-	-	0.00	0.00
<i>Penicillium citrinum</i>	Mean	0.00	9.33*	0.00	18.53
	Std. Deviation	0.00	0.51	0.00	0.55
	Lsd Sig.	-	0.00	-	0.00
<i>Aspergillus flavus</i>	Mean	0.00	0.00	5.03*	12.06
	Std. Deviation	0.00	0.00	0.25	0.90
	Lsd Sig.	-	-	0.00	0.001
<i>E.coli</i>	Mean	14.06*	7.06	6.03	20.50
	Std. Deviation	0.11	0.90	0.25	1.04
	Lsd Sig.	0.00	-	-	0.00
<i>Staph. aureus</i>	Mean	12.26*	9.36	8.00	21.81
	Std. Deviation	0.47	0.11	0.20	0.15
	Lsd Sig.	0.00	-	-	0.000
<i>Salmonella typhi</i>	Mean	13.06*	0.00	0.00	25.06
	Std. Deviation	0.35	0.00	0.00	0.95
	Lsd Sig.	0.00	-	-	0.00
<i>Erwinia carotovora</i>	Mean	0.00	7.33	8.00*	17.30
	Std. Deviation	0.00	0.25	1.00	0.26
	Lsd Sig.	-	-	0.00	0.00
<i>Streptococcus sp.</i>	Mean	0.00	0.00	9.60*	20.00
	Std. Deviation	0.00	0.00	0.10	0.30
	Lsd Sig.	-	-	0.01	0.00

*. The mean difference is significant at the p < 0.05 level. /POSTHOC=LSD ALPHA(0.05).

The results presented in table 4 showed the effect of water fruiting bodies crude extracts of mushrooms against the tested pathogenic bacteria and fungi. It was found that the water extract of G7 was significantly effective against *Fusarium solani* that give inhibition zone (11.0mm) followed by G3 water extract recorded inhibition zone (10.06mm). It is clear that H2O extract of G3 and G7 are more effective than ethylacetate and methanol extracts against *Fusarium solani*

Water extracts of G3 and A40 have again the same effect against *Curvularia sp* with inhibition zones 11.0 & 11.06mm respectively, but lower than their ethylacetate extracts. The water extract of both G3 and A40 were significantly effective against *Rhizoctonia solani* and recorded inhibition zones 10.0mm for each, but G7 did not show activity. The water extract of A40 was significantly effective against *Fusarium oxysporum* that give inhibition zone (17.0mm) followed by G7 (11.3mm) which was the opposite effect of their ethylacetate extracts, but G3 hasn't any effect. It is clear that the effect of water extract of A40 was higher than its ethyl acetate and methanolic extracts on *F. oxysporum*. Water extract of A40 was significantly effective against *Penicillium italicum* that gives inhibition zone (8.0mm) followed by G7 water extract (7.06mm), but G3 did not show activity. Water extract of G7 was

significantly effective against *Penicillium citrinum* and recorded inhibition zone (12.0mm). The same effect of A40 mycelia, ethylacetate, methanolic and water extracts was noticed against *Aspergillus flavus* that give inhibition zone (5.066, 4.03, 5.03 & 4.03mm respectively). Water extract of G3 was significantly effective against *E.coli* and give inhibition zone (12.06mm) whereas, G7 and A40 water extracts were not significantly effective against *E.coli* and recorded inhibition zones 9.0 & 7.0mm respectively. water extract of G3 was significantly effective against *Staphylococcus aureus* that give inhibition zone (13.03mm) followed by G7 and A40 water extracts that give inhibition zone (9.06, 9.0mm). Water extract of G7 was significantly effective against *Erwinia carotovora* that give inhibition zone (13.06mm) followed by A40 water extract that give inhibition zone (10.03mm), but G3 water extract did not show any effect. On the other hand, water extracts of G3 only was effective against *Salmonella typhi* as shown in tables 1, 2 and 3 that give inhibition zone (14.03mm). Water extract of A40 was the only mushroom effective against *Streptococcus sp.* which gives inhibition zone (10.0mm). Mushroom extracts have a lower antimicrobial activity as compared with antifungal (nystatin) against all micro-oraganisms except ethylacetate extracts of G3 and A40 against *Curvularia sp.*

Table 4. the antimicrobial effect of water fruiting bodies crude extracts of A40, G3 and G7 against tested pathogen. Inhibition zone represented by (mm):

Tested organism		H2O extracts			
		G3	G7	A40	(nystatin/pencillin)
<i>Fusarium solani</i>	Mean	10.06	11.00*	0.00	21.03
	Std. Deviation	0.95	0.20	0.00	0.05
	Lsd Sig.	-	0.00	-	0.001
<i>Curvularia sp.</i>	Mean	11.00*	0.00	11.06*	15.03
	Std. Deviation	0.45	0.00	1.00	1.05
	Lsd Sig.	0.001	-	0.00	0.00
<i>Rhizoctonia solani</i>	Mean	10.00*	0.00	10.00*	16.00
	Std. Deviation	0.40	0.00	0.26	0.76
	Lsd Sig.	0.00	-	0.00	0.01
<i>Fusarium oxysporum</i>	Mean	0.00	11.30	17.00*	21.00
	Std. Deviation	0.00	0.10	0.26	0.45
	Lsd Sig.	-	-	0.00	0.02
<i>Penicillium italicum</i>	Mean	0.00	7.06	8.00*	17.00
	Std. Deviation	0.00	0.95	0.10	1.00
	Lsd Sig.	-	-	0.00	0.00
<i>Penicillium citrinum</i>	Mean	0.00	12.00*	0.00	18.53
	Std. Deviation	0.00	0.20	0.00	0.55
	Lsd Sig.	-	0.00	-	0.00
<i>Aspergillus flavus</i>	Mean	0.00	0.00	4.03*	12.06
	Std. Deviation	0.00	0.00	0.06	0.90
	Lsd Sig.	-	-	0.00	0.001
<i>E.coli</i>	Mean	12.06*	9.00	7.00	20.50
	Std. Deviation	1.00	0.20	0.40	1.04
	Lsd Sig.	0.001	-	-	0.00
<i>Staph. aureus</i>	Mean	13.03*	9.06	9.00	21.81
	Std. Deviation	0.06	0.90	0.26	0.15
	Lsd Sig.	0.00	-	-	0.00
<i>Salmonella typhi</i>	Mean	14.03*	0.00	0.00	25.06
	Std. Deviation	0.25	0.00	0.00	0.95
	Lsd Sig.	0.00	-	-	0.00
<i>Erwinia carotovora</i>	Mean	0.00	13.06*	10.03	17.30
	Std. Deviation	0.00	1.00	0.05	0.26
	Lsd Sig.	-	0.00	-	0.00
<i>Streptococcus sp.</i>	Mean	0.00	0.00	10.00*	20.00
	Std. Deviation	0.00	0.00	0.10	0.30
	Lsd Sig.	-	-	0.01	0.00

*. The mean difference is significant at the p < 0.05 level. /POSTHOC=LSD ALPHA(0.05).

G3 extracts (ethylacetate, methanol, water) were most effective against pathogenic bacteria as *E.coli*, *Staphylococcus aureus* and *Salmonella*. Similar study was reported by Sheena *et al.* (2003) who observed antimicrobial activity of *G. lucidum* against *Salmonella*, *E.coli*, and *Bacillus subtilis* species. *Ganoderma* samples were highly active against gram negative as well as gram positive bacteria with a broad spectrum antibiotic. Prasad and Wesely (2008) reported that methanolic extract of *Ganoderma lucidum* from India have efficient antimicrobial effect against MRSA. Klaus & Niksic, (2007) reported the effect of different extracts isolated from *Ganoderma lucidum* on *Staphylococcus aureus*, *E. coli*, *Bacillus*, and *Salmonella* species. Extracts from *G. pfeifferi* (Mothana *et al* 2000) and *G. applanatum* (Smania *et al* 1999) have been shown to possess important antibacterial effect against *E.coli*. Ethylacetate and water extracts of G7 were effective on *Erwinia carotovora* also, Water extract of A40 was effective on *Erwinia carotovora*. A40 extracts were the most effective against *Streptococcus sp.*, and *Aspergillus flavus*, whereas G7 and G3 extracts were not effective. G7 extracts were the most effective against *Penicillium citrinum*, whereas A40 and G3 extracts were not effective. G3 ethylacetate extract was more effective on *Fusarium solani*, *Curvularia sp.*, *Rhizoctonia solani* than its methanolic and water extracts. Water extract of G7 was more effective on *Fusarium solani*. The antimicrobial screening of different solvent extracts of *G. lucidum* on some species of bacteria was investigated by Ofodile and Bikomo (2008), some bioactive components such as alkaloids, terpenoids, and phenolics were implicated to be the reason for the effect of fruit bodies and mycelial culture of *G. lucidum*. Sheena *et al.* (2003) reported the potential antibacterial effect of three medicinally important mushrooms including *Ganoderma lucidum*, in India. Yang *et al.*, (2002) investigated that many antimicrobial compounds such as polysaccharides, lectins, terpenes etc. act on the cytoplasmic membrane of bacteria. The activity of extracts from mycelia and fruiting body of *G. lucidum* against clinical bacteria in Asia have also been investigated (Gao *et al.*, 2003). Zjawiony (2004) showed that 75% of tested polypore fungi have strong antimicrobial effects. Mushroom based products either from fruiting bodies and the mycelia are consumed in the form of extracts, capsules, or tablets (Filipa *et al.*, 2013). The spectrum of detected biological activities of macrofungi is very broad that necessary for use as a food supplements, drug or other purpose is the continuous production of mycelium in high amounts and in a standardized quality. The indiscriminate use of antibiotics has developed antibiotics resistance common bacterial pathogens, which created immense clinical problems in the treatments of diseases. Therefore, there is a need to search for nontoxic, non-antibiotic plant based alternative. Other *Ganoderma* species as *Ganoderma lucidum* have been used in combination with chemotherapeutic agents to treat different bacterial diseases (Gao *et al.*, 2003). There has been increased interest in the use of natural substances as food preservatives and antioxidants, due to toxicological concerns associated with the usage of synthetic substances in food and increasing awareness about natural foods. Although Biag *et al.*, (2015) recorded the inhibition

activity of *G. lucidum* against *A. flavus* but in our results there was no evidence that any of the three *Ganoderma* spp exhibited any effect on it.

Mushroom species as *Agaricus*, *Lentinus*, *Pleurotus*, *Russula*, and *Cantherellus* showed some antimicrobial activity (Dulger *et al.*, 2004; Aziz *et al.*, 2007 and Manjunathan *et al.*, 2010). Mushrooms species have different constituents with different concentrations that has different antimicrobial activities and result from genetic structure of mushroom species as suggested by researchers reported the antimicrobial effect of *Agaricus bisporus*, *Pleurotus spp.*, and *T. boudieri* (Uzun *et al.*, 2004; Demirhan *et al.*, 2007; Iwalokun *et al.*, 2007 and Jagadish *et al.*, 2008). In this study, water extract with high antimicrobial activity in some organisms due to the most of the phenolic compounds are soluble in water. In this study, ethylacetate extract has antimicrobial activity as most active components are water insoluble, so, low polarity organic solvents yield more active extracts (Cowan, 1999). Dulger and Gonuz (2004) showed the antimicrobial properties of four different extracts of mushroom (*Cantharellus cibarius*) against fifty important human pathogens. Polysaccharide components were found to be the principle bioactive that plays a significant role in antibacterial effect (Yoon *et al.*, 1994). Both strains of *Agaricus* showed inhibition activity against Gram +ve and Gram -ve which disagree with Öztürk *et al.*, (2011) who reported that *A. bitorquis* are most efficient on Gram +ve bacteria. Rana *et al.*, (2008) suggested that *A. bitorquis* exhibited antagonistic effect on *B. subtilis* with weakly affected the other bacteria tested.

CONCLUSION

The major finding of the study that the extracts of some Egyptian wild *Agaricus* and *Ganoderma* species proved significant and antimicrobial activities against some pathogenic bacteria and fungi. They could play an important role in developing low cost and better quality drugs.

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تقييم الانشطة المضادة للميكروبات بالغزل الفطري و بالمستخلصات الخام لبعض انواع عيش الغراب البري المصري مثل انواع الاجاريكس والجانودرما فاطمه فتحى مجاهد ، اميرة علي الفلال و سمر سعد الشوبكي قسم النبات ، كلية العلوم ، جامعة المنصورة ، مصر البريد الالكتروني: semsemaad89@yahoo.com

تركز الدراسة الحالية على تقييم الانشطة المضادة للميكروبات لبعض انواع عيش الغراب البري المصري : سلالتين من الاجاريكس ((*bisporus Agaricus*) (60A و 40A) (*bitorquis Agaricus*) وثلاثة انواع من الجانودرما (7G,3G,1G) تم التحقيق معهم ضد 13 كائن حي دقيق مسبب للأمراض (ثمانية انواع فطريه ممرضة و خمسة انواع بكتيرية ممرضة) وكان الغزل الفطري ل ((7G ,3G ,40A اكثر فاعلية على اكبر عدد من الكائنات الحية الدقيقة المسببة للأمراض. تم فحص الانشطة المضادة للميكروبات بالمستخلصات الخام من ((7G ,3G ,40A بواسطة مذيبات مختلفة (الميثانول ، الماء ، الايثانيل اسيتات) بواسطة طريقة الانتشار في الاجار من خلال حفرة (Agar well diffusion). اظهرت النتائج ان هذه الفطريات من عيش الغراب لها انشطة فعالة كمضادات للفطريات ومضادات للبكتيريا. تم عرض التأثير العدائي الاعلى لمستخلصات عيش الغراب بواسطة مستخلص ايثانيل اسيتات من 7G ضد *oxysporum Fusarium* و 3G هو الاكثر فاعلية ضد السالمونيلا التيفيه. مستخلصات عيش الغراب لها انشطة مضادة للفطريات ومضاده للبكتيريا اقل عندما تقارن بمضاد للفطريات ماعدا مستخلصات الايثانيل اسيتات لكل من 3G و 40A اظهرت تأثير تثبيط اعلى ضد *sp Curvularia*.