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COMBINATION ANTIMICROBIAL EFFICACY BETWEEN THE MUSHROOM (*PLEUROTUS OSTREATUS*) AND SOME COMMERCIAL ANTIBIOTICS

Tahany M.A. Abd El-Rahman, Botany and Microbiology Department, Faculty of Science, Cairo University

Rasha M. Naguib, Micro Analytical Center, Faculty of Science, Cairo University

Sayed Mohamed Sayed, Botany and Microbiology Department, Faculty of Science, Cairo University

Alaa M. Saleh, B. Sc. Student, Biotechnology and Biochemistry program

Aliaa M. Alaa, B. Sc. Student, Biotechnology and Biochemistry program

Amina F. Ali, B. Sc. Student, Biotechnology and Biochemistry program

Fatma R. Waly, B. Sc. Student, Biotechnology and Biochemistry program

Linah M. Hussein, B. Sc. Student, Biotechnology and Biochemistry program

Abstract: S*usceptibility assays were carried out using four antibacterial and four antifungal commercial drugs against four pathogenic bacterial species and one pathogenic fungal species. The activity was compared with the antimicrobial activity of P. ostreatus mycelia extract. The results indicated high antimicrobial activity of mycelial extract against B. subtilis, E. coli, S. aureus and P. aeruginosa. B. subtilis was found to be the most susceptible to mycelial extract.*

Ampicillin, Augmentin, Norfloxacin and Tetracycline exhibited lower activities than that of the mycelial extract against the pathogenic bacteria. The antifungal activity of mycelial extract against C. albicans detected lower values than that of Fluconazole, Amphotericin B, Terbinafine except Griseofulvin which achieved no antifungal activity on C. albicans. Antimicrobial assay of combination between P. ostreatus mycelial extract with the antibacterial Norfloxacin or the antifungal Terbinafine against B. subtilis or C. albicans, respectively, declared that macrodilution method is more sensitive than agar diffusion method in MIC determination. The tenfold dilution is more accurate than the bifold dilution in combination experiment. Three synergistic mixtures were explored in this study, Norfloxacin + P. ostreatus mycelial extract against B. subtilis (FICI=0.011) using macrodilution method; Norfloxacin + P. ostreatus mycelial extract against B. subtilis using agar diffusion method (FICI=0.11) and P. ostreatus mycelial extact + Terbinafine against C.

albicans using macrodilution method (FICI=0.10001). These synergistic mixtures could be used in many technological applications include medicinal, pharmaceutical and food industries. This study also described that the antioxidant activity, protein and total phenol contents in P. ostreatus were higher than in carrot root used as standard plant.

Key words: *Pleurotus ostreatus, Antibacterials, Antifungals, Combination Therapy, Pathogenic Bacteria, Pathogenic Fungi*

INTRODUCTION

Investigators are currently explored several approaches for controlling pathogenic bacteria and fungi including antibiotics, probiotics, vaccines and bacteriophages. Increasing bacterial and fungal resistance to antibiotics, in addition to increasing concerns about the significant drawbacks of systemic additives has led to wider public acceptance of natural compounds with their lower level of toxicity and fewer negative environmental hazards (Tajkarimi and Ibrahim, 2011). Recently, global antimicrobial resistance is an increasing public health problem, so different plant products have considered to be an important therapeutic agents in many fields such as antimicrobials, anticancer and antioxidants. The current widespread belief that "Green medicine" is safe and more dependable than the costly synthetic drugs with many adverse side effects (Nair and Chanda, 2007).

Mushrooms have long been used as a valuable food source and as traditional medicine around the world since ancient times (Nair and Chanda, 2007; Cordell, 2000; Wasser and Weis, 1999; Sagakami et al.1991). Both fruiting body and mycelium contain compounds with antimicrobial activity and a number of medicinal mushrooms such as *Coprinus*, *Pleurotus*, *Polysporus* and *Poria* are rich in β-glucan, lectin, phenolic compounds, flavonoides, polysaccharides, terpenoids, fibers, tannins, lovastatin, steroid, glycopeptides, terpenes, saponins, coumarins, alkaloids and purin (Benedict and Brady, 1972; Conchran et al.1978; Karacsonyi and Kuniak, 1994; Collins and Ng, 1997; Smania et al.2003; Wang et al.2004; Cohen et al.2002). *P. ostreatus* mushrooms are rich source of natural antibiotics where the cell wall glucans have immunomodulatory properties in addition to many external secondary metabolites which inhibit growth of bacteria, fungi and viruses (Benedict and Brady, 1972; Suzuki et al.1990; Collins and Ng, 1997; Gao et al.2005). *P. ostreatus* is now grown commercially around the world for food and medicine. It has low calories (each100g have 28kcal) and low sodium. It used for many years in fold medicine. The Na/K ratio is

suitable for people with hypertension, diabetes and obesity (Miguel et al.1997). To date, approximately 70 species of *Pleurotus*, commonly known as oyster mushroom, have been recorded. Most of these species exhibit antimicrobial, haematological, antiviral, antitumor and immunomodulatory activity (Aqil et al.2006; Akyuz and Kirbag, 2009).

Combination therapy by more than one drugs based on i) The mechanism of action (combining agents with complementary targets with the microbial cells), ii) Spectrum of activity (combining agents potent against different microorganisms), iii) Stability and pharmacokinetics/pharmacodynamics characteristics and iv) Reduction in the number of resistant organisms(Bennett et al.1979). Many advantages have been reported on the combination therapy such as broad spectrum of activity, great potency than either of the drugs used in monotherapy, improved safety anddurability by reducing the dose of antimicrobial drug, reduce the mutant development, reduce the microbial resistance and shorten the time course of treatment. The aim of this study was to evaluate the antimicrobial efficacies of *P. ostreatus* as compared with some widely used antibiotics in Egypt. Single and combined treatments were assayed against some human bacterial and fungal pathogens using different techniques. Antioxidant activity, protein content and total phenols were estimated and compared with carrot used as standard.

MATERIALS AND METHODS

Mycelial culture: *Pleurotus ostreatus* culture was kindly supplied by City of Science and Technology, Alexandria, Egypt.

Microorganisms: The tested microorganisms were obtained from culture collection in microbiology lab in Botany and Microbiology Department, Faculty of Science, Cairo University. Gram positive: *Bacillus subtilis* CMGB 215, *Staphylococcus aureus* ATCC 6588; Gram negative: *Escherichia coli* $CBAB_2$, *Pseudomonas aeruginosa* ATCC 15442 and yeast fungus *Candida albicans* ATCC 20231. All microorganisms are human pathogens.

Antibiotic discs: Includes, Norfloxacin(10µg/disc), Tetracycline(10µg/disc), Ampicillin(10µg/disc), Augmentin(10µg/disc), Fluconazole(10µ/disc), Amphotericin B(10µg/disc), Terbinafine(10µg/disc), Griseofulvin(10µg/disc). The discs were prepared by saturating the discs in antibiotic immersions, air dried and immediately used in assay tests. All antibiotics were purchased from the local pharmacies in Egypt.

Extraction of bioactive compounds from *P.ostreatus* mycelium: The mycelia were maintained on potato dextrose agar(PDA) at 4^{o}C and subcultured at regular interval to maintain it viable. The fresh young mycelium at the early stage of growth (3 days old) was immersed in the extraction mixture composed of methonal: glycerol: water(1:1:1 v/v). The process of extraction took place for one week in dark place at 10^{o}C using orbital shaker at 150 rpm (Pauliuc and Dorica, 2013). The extract was then filtered, concentrated by using a rotavap and weighed. The dry material extract was diluted by distilled water for the testes.

Susceptibility test: It was performed using agar diffusion method as recommended by the Clinical and Laboratory Standard Institute(CLSI, 2003).Microbial suspension 100µl was spread onto the agar plates then antibiotic discs were placed onto the surface of each plate using a sterile forceps. After incubation at 37^{o}C for 24h, the diameter of inhibition zones were measured. In case of mycelial extract of *P.ostreatus* filter paper discs were saturated with the extract, then air dried and placed onto the surface of the inoculated plates(Bauer et al. 1966). The plates incubated as previously described and the inhibition zone diameter measured.

Combination assay of the antimicrobial activity: Between the mycelial extract and the commercial antibiotics, Agar diffusion method was applied. A Muller-Hinton agar plates were inoculated with the bacterial suspension then the antibiotic discs were loaded with 100µl of mycelial extract and placed on the surface of inoculated plates to see the combined activity. The plates incubated and 37^{o}C at then the inhibition zone diameters were measured.

Determination of minimum inhibitory concentration(MIC): Bifold serial dilution(20-2500) and tenfold serial dilution(10^{-6}-10^{-1}) were prepared. In addition, agar diffusion plate method(previously described) and macrodilution tube methods were used in MIC determination. Macrodilution tube method(Alade and Irobi, 1993),each dilution of either mycelial extract(1.0ml) or antibiotic(1.0ml) or their combination(1:1 v/v) were dispersed into test tubes containing 5ml of nutrient broth for *B.subtilis* or Potato dextrose broth for *C.albicans*.Each bacterial or fungal species(100µl of 10^{8}CFU/ml) was inoculated into the test tubes. The tubes mixed and incubated as previously described. The tubes then examined for microbial growth. MIC was defined as the minimum concentration of mycelial extract or

antibiotic or both in combination that did not allow any visible growth or turbidity in the tubes.

Determination of total phenols: The total phenolic compounds in mycelial extract of *P.ostreatus* were spectrophotometrically determined at 750nm as described by Lee et al.(2001) using Folin-Ciocateau phenol reagent. Standard solution of gallic acid (20-200µg/ml) was prepared to use in standard curve. The total phenol in mycelial extract was expressed as µg/ml of gallic acid equivalent.

Determination of protein content: In mycelial extract of *P. ostreatus*was carried out using the method of(Lowry et al. 1951).

Antioxidant activity assay: The antioxidant activity was assayed using(1,1-Diphenyl-2-picryl-hydrazyl"DPPH") Scavenging assay (Vani et al. 1997),this method is based on the reaction of the colored free radicals in the DPPH with the mycelial extract. The color of the DPPH turns from purple to yellow and the molar absorptivity of the DPPH radical is measured against a blank at 517nm(Aruna et al. 2001). The inhibition activity of the radicals was calculated as (%) in the following way: % scavenging activity = $\{(A_{blank} - A_{sample}) / A_{blank}\} \times 100$

Elucidation of the bioactive groups: In *P.ostreatus* mycelial extract was carried out by using FTIR.

RESULTS AND DISCUSSION

Antibacterial assays were done on each of *P.osteatus* mycelial extract and some of the commercial antibiotics in Egypt against *B.subtilis*, *E.coli*, *S.aureas* and *P.aeruginosa*. The data in table1 revealed that mycelial extract of *P.ostreatus* exhibited significant antibacterial activity against the tested pathogens particularly *B.subtilis* and *P.aeruoginosa* with 33.3mm and 29mm inhibition zone, respectively. Norfloxacin showed high activity against *B.subtilis*; Augmentin and Tetracycline against *P.aeuroginosa*; and Ampicillin against *E.coli*. The relative activity of antibiotics in comparison with the mycelial extract revealed higher antibacterial efficacy of the mycelial extract over the commercial antibiotics by 104% to 199% in almost all cases(Fig.1).

Candida albicans, however, showed significant resistance to the mycelial extract with 8.6mm inhibition zone(Table2). The relative activity indicated high decrease in mycelial extract antifungal activity than that of Fluconazole, Amphotericin B & Terbinafine by 29.0%, 57.3% and 26.9%, respectively. Griseofulvin showed less activity than that of mycelial

extract. In conclusion *P.ostreatus* mycelial extract exhibited high antibacterial activity but low antifungal activity. Norfloxacin and Terbinafine were the most potent antibiotics against *B.subtilis* and *C.albicans*, respectively. In close relation with us Akyuz et al.(2010) reported that *P.ostreatus* and other mushrooms were found to have antimicrobial activities against *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *C. albicans*, *C.glabrata*, *Trichophyton* spp. and *Epidermophyton* spp. These antimicrobial activities have lower effect than the antibiotics Nystatin and Streptomycin sulfate. Similarly, *P.ostreatus* extract strongly inhibited the growth of *B.cereus*, *Listeria innocua*, *P.aeuroginosa*, *S.aureas* and *C.albicans*(Vamanu, 2012).Furthermore, Iwalokum et al.(2007) found that organic extracts of *P.ostreatus* were effective against 89.8% of the tested microbial isolates with *B.subtilis*, *E.coli* and *saccharomyces cerevisae* exhibited the highest susceptibilities. Gemmotherapic extract of *P.ostreatus* had significant inhibitory activity against *B.subtilis*, *B.cereus* var mycoides and *Serratiamarcesceus* (Paulic and Dorica, 2013).

Table1: Antibacterial activity of *P.ostreatus* mycelial extract in comparison with some commercial antibiotics against pathogenic bacteria

Bacterial pathogens	Ampicillin		Augmentin		Norfloxacin		Tetracycline		Mycelial ExtractA (mm)
	A(mm)	R.A (%)	A (mm)	R.A (%)	A (mm)	R.A (%)	A (mm)	R.A (%)	
B. subtilis	24.6± 0.231	(+) 136	22± 0.101	(+) 152	29.3± 0.461	(+) 114	26.3± 0.24	(+) 127	33.3± 0.611
E.coli	25± 0.665	(+) 114	27.3± 0.561	(+) 104	19.6± 0.111	(+) 145	18± 0.121	(+) 158	28.3± 0.440
S.aureus	14.6± 0.401	(+) 199	18.3± 0.623	(+) 159	23± 0.222	(+) 126	19± 0.131	(+) 153	29± 0.311
P.aeruginosa	19.6± 0.111	(+) 138	28.6± 0.233	(-) 95	25.3± 0.442	(+) 107	29.6± 0.415	(-) 92	0.27± 0.232

- A: Activity: Inhibition zone diameter (mm),R.A: Relative activity = (A. of mycelial extract / A. of antibiotic) x 100, (+) higher A. of mycelial extract than antibiotic, (-) lower A. of mycelial extract than antibiotic.

Table2: Antifungal activity of *P.ostreatus* mycelial extract in comparison with commercial antifungal and their relative activities

Fungal pathogen	Fluconazole		Amphotericin B		Terbinafine		Griseofalvin		Mycelial ExtractA (mm)
	A (mm)	R.A (%)	A (mm)	R.A (%)	A (mm)	R.A (%)	A (mm)	R.A (%)	
C.albicans	29.0± 0.322	(-) 29.6	15.0± 0.131	(-) 57.3	32.0± 0.222	(-) 26.9	1.1± 0.213	(+) 781.8	8.6± 0.0013

- A: Activity: Inhibition zone diameter(mm), R.A: Relative activity= (A of mycelial extract / A of antifungal) x 100,(+) higher A. of mycelial ext. than antifungal,(-) lower A. of mycelial ext. than antifungal.

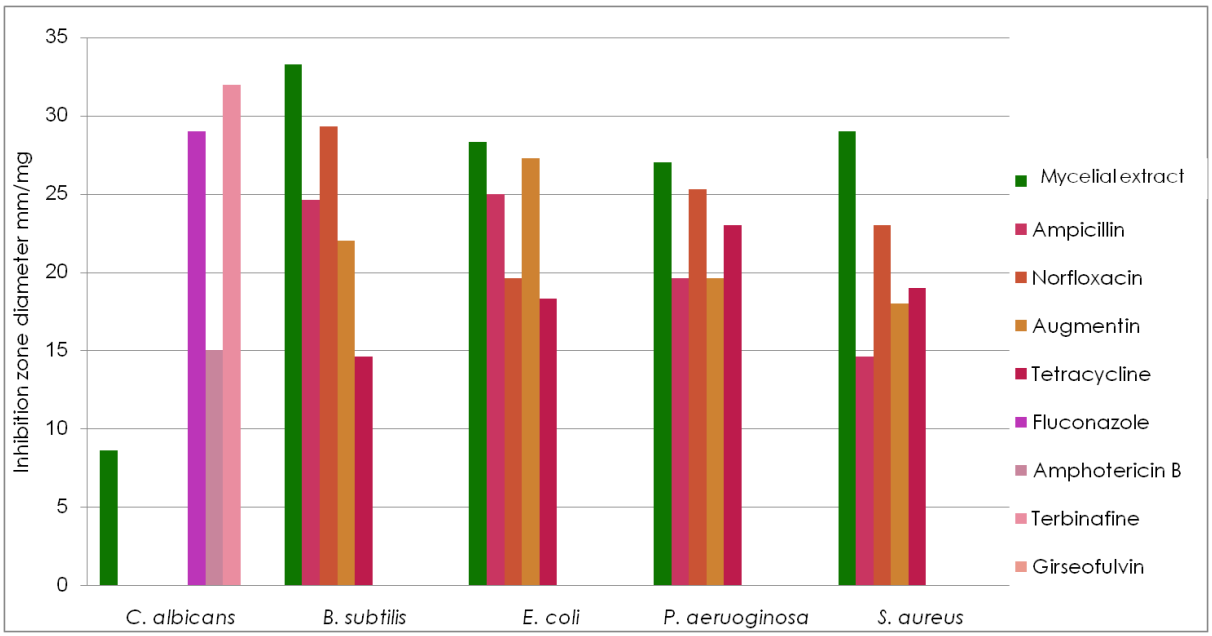


Figure 1: Comparison between activities of the *P. ostreatus*mycelial extract with the antimicrobial drugs in Egyptian markets

By comparison between commercial antibiotics and the mycelial extract of *P. ostreatus* on the tested microorganisms the data (Fig. 1) indicated that mycelial extract exhibited higher antimicrobial activity against all tested microorganisms except *C. albicans*.

Determination of MIC of Norfloxacin, Terbinafine, and *P. ostreatus* mycelial extracttested singly or in combination using Bifold dilution method:

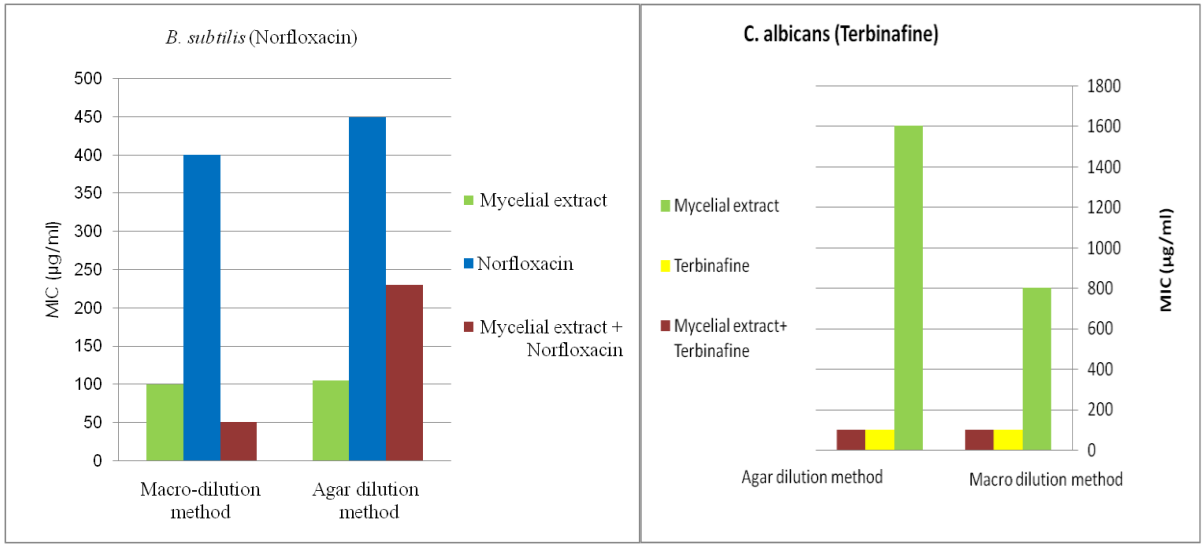


Figure 2: MIC values (µg/ml) of Norfloxacin, Terbinafine, and *P. ostreatus* mycelial extract when treated singly or in combination against *C. albicans* and *B. subtilis* using Bifold dilution method

Macrodilution method:

The data in Figure2 indicated that:Norfloxacin alone was less effective against *B. subtilis* (MIC: 400µg/ml) than *P. ostretus* mycelial extract (MIC: 100µg/ml).Combination between the two agents led to further increase in effectiveness and reduced the MIC to 50µg/ml.In case of *C. albicans* the antifungal agent Terbinafine when added singly, exerted much more effectiveness (MIC: 100µg/ml) than *P. ostretus* mycelial extract which recorded high MIC value (800µg/ml).Combination between the two agents reduced the MIC to the level of Terbinafine alone (MIC: 100µg/ml).

Agar dilution method:

In *B. subtilis* the Agar dilution method proved to be less sensitive than Macro-dilution method as the MIC raised in all cases than that in Macro-dilution method.

Norfloxacin was the least antibacterial agent in its effectiveness (MIC: 450µg/ml) while *P. ostretus* mycelial extract was tightly potential agent (MIC: 105µg/ml). Combination between two agents increase the effectiveness (MIC: 225µg/ml) than Norfloxacin alone but it still lower than that of the mycelial extract.

In case of *C. albicans*, *P. ostreatus* mycelial extract achieved very low antifungal activity (MIC: 1600µg/ml), while Terbinafine was highly active against *C. albicans*. Combination mixture between the two agents exerted activity equal to that of Terbinafine alone (MIC: 100µg/ml each).

Determination of FICI for Bifold dilution method:

Table 3: FICI of the interactions between Norfloxacin or Terbinafine with P. ostretus mycelial extract against *B. subtilis* and *C. albicans* using bifold dilution method

Bifold dilution	FICI			
	***B. subtilis* (Norfloxacin)**		***C. albicans* (Terbinafine)**	
	Macro-dilution method	**Agar dilution method**	**Macro-dilution method**	**Agar dilution method**
FICI of the Combination	0.625	2.70	1.125	1.063
Type of interaction	No interaction	No interaction	No interaction	No interaction

- FICI:Functional Inhibitory Concentration Index.
- $FICI = FIC_A + FIC_B$

$$= \frac{\text{MIC in combination}}{\text{MICA tested alone}} + \frac{\text{MIC in combination}}{\text{MICB tested alone}}$$

- FICI ≤ 0.5: Synergy (S).
- 0.5 < FICI ≤ 4.0: Indifference
- FICI > 4.0 Antagonism (A).

The data in Table (3) revealed that on using bifold dilution method, we didn't detect any synergistic or antagonistic mixtures. In the combination between the commercial antibiotics and *P. ostretus* mycelial extract.The FICI values were higher than 0.5 and lower than 4, which means no interaction or indifferent effect in all cases.

Determination of MIC values (µg/ml) of Norfloxacin, Terbinafine, and P. ostreatus mycelial extract tested singly or in combination by Tenfold dilution method:

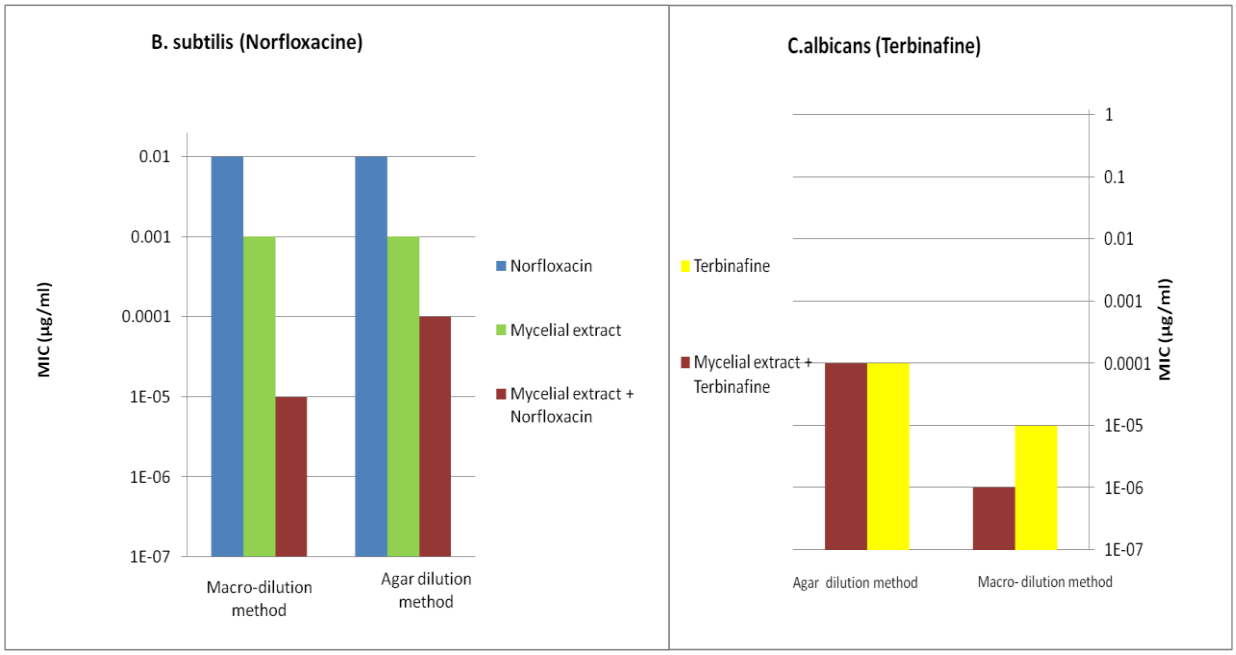


Figure 3: MIC values (µg/ml) of Norfloxacin, Terbinafine, and *P. ostreatus* mycelial extract when treated singly or in combination against *B. subtilis and C. albicans* using Tenfold dilution method

Macrodilution method:

The data in Figure3 revealed that in using,tenfold dilution method the commercial antibiotic Norfloxacin had the least effectiveness against *B. subtilis* (MIC: 10^{-2} dilution), followed by *P. ostreatus* mycelial extract (MIC 10^{-3}dilution).Combination between the two agents led to further increase in effectiveness with lower MIC value (10^{-5} dilution).

In *C. albicans* the MIC of *P. ostreatus* mycelial extract could not be detected at all experimental dilutions, which revealed that it is inactive against*C. albicans.* Terbinafine, however, was effective against *C. albicans* with (MIC 10^{-5} dilution).Combination between the two agents exerted higher antifungal potentiality with (MIC 10^{-6} dilution).

Agar dilution method:

Norfloxacin was the least active agent against *B. subtilis* (MIC 10^{-2} dil.) followed by *P. ostretus* mycelial extract with (MIC 10^{-3} dil.).Combination between the two agents led to further increase in effectiveness against *B. subtilis* with (MIC 10^{-4} dil.).

In case of *C. albicans*, *P. ostretus* mycelial extract was not active and the MIC not achieved within the tested dilutions.Terbinafine was effective against *C. albicans* with (MIC 10^{-4} dil.) Combination between the two agents (Terbinafine and *P. ostretus* mycelial extract) had the same effectiveness as Terbinafine alone with the same MIC value (10^{-4} dil.).

Determination of FICI for Tenfold dilution method:

Table 4: Determination of FICI of interactions between Norfloxacin or Terbinafine with *P. ostretus* mycelial extract against *B. subtilis* and *C. albicans*

Tenfold dilution	FICI			
	***B. subtilis* (Norfloxacin)**		***C. albicans* (Terbinafine)**	
	Macro-dilution method	**Agar dilution method**	**Macro-dilution method**	**Agar dilution method**
FICI of the Combination	0.011	0.11	0.10001	1.01
Type of interaction	Synergism	Synergism	Synergism	No interaction

- FICI: Functional Inhibitory Concentration Index.
- $FICI = FIC_A + FIC_B$

$$= \frac{\text{MIC in combination}}{\text{MICA tested alone}} + \frac{\text{MIC in combination}}{\text{MICB tested alone}}$$

- FICI ≤ 0.5: Synergy (S).
- 0.5 < FICI ≤ 4.0: Indifference
- FICI > 4.0 Antagonism (A).

It was clearly evident from table (4) that we could achieve three synergistic mixtures in this experiment which were:

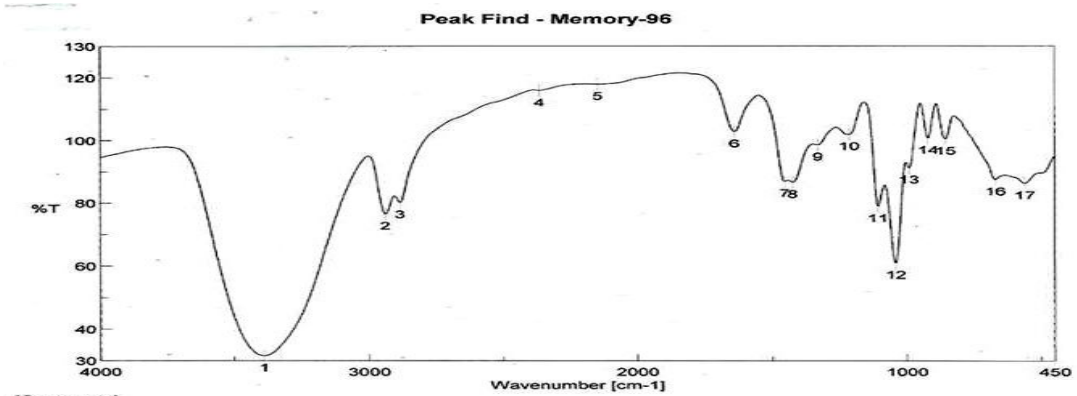
- Norfloxacin + *P. ostretus* mycelial extract against *B. subtilis* (FICI = 0.011) by using Macro-dilution method.
- Norfloxacin + *P. ostretus* mycelial extract by using Agar diffusion method against *B. subtilis*.
- Terbinafine + *P. ostretus* mycelial extract against *C. albicans* by using Macro-dilution method.

Those synergistic mixtures mean that: The effectiveness increased than the single treatment.The dose of antibiotic used decreased to half concentration.The toxicity

reduced.The resistance of pathogens toward antibiotics reduced due to the difference in mechanism of action of both compounds.

In this respect Mokherjee et al. (2005) claimed that single treatment of microbial infection is often complicated by high toxicity, low tolerability or narrow spectrum of activity. These difficulties have driven recent effort to determine the efficacy of combination therapy in the treatment and management of invasive infection. In vitro susceptibility assays are highly dependent on the microbial species under investigation and on the assay condition such as incubation temperature, exposure time, media and other specific factors(Kirkpatrick et al.2002; Lewis et al.2002; Mencacci et al.2000). The antimicrobials in combination are dependent not only on drug-drug interaction or concentration but also on the ratio of drugs(Ghannoum et al.1989; Ghannoum et al.1995; Ghannoum and Elewski,1999). However, Mossave and Shavis(2013) found that Nisin did not inhibit *E.coli* 0157:H7 while essential oil of *Mentha spicata*inhibited it with MIC value of 40µl/ml. Combination of both compounds increase the antibacterial efficacy and might be a potential source of preservative for the control of E.coli in the food industry.

Instrumental analysis of the main groups in *P. ostreatus* mycelial extract



[Comments]
Sample name 63.611
Comment 23/3/2016
User IR
Division IR
Company MAC

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3389.28	31.5978	2	2938.98	76.5419	3	2884.99	80.1854
4	2364.3	115.847	5	2147.35	117.868	6	1640.16	102.738
7	1455.03	86.9486	8	1425.14	86.6169	9	1332.57	98.4989
10	1215.9	101.927	11	1107.9	78.9627	12	1042.34	60.9741
13	993.16	91.1962	14	920.843	100.644	15	856.239	100.38
16	670.142	87.5664	17	561.184	86.2578			

Figure 4: The FTIR spectrum of the mycelial extract of *P. ostreatus*

Figure 4 showed that the spectrum possess characteristic absorption peaks for polysaccharides. The strong peak at 3389.28 cm^{-1}(1) is the absorption of the O-H bond. Peaks 2 and 3 at spectrum 3936.98 and 2884.99 indicated the presence of uronic acid.The

weak peak at 2364 cm^{-1} (4) showed the absorption of C-H bond. The peak at 1640.16 cm^{-1}(6) is the hydrated water in the polysaccharide. The peaks from1215.9cm^{-1} to 1034.34 cm^{-1}showed the absorption of C-O bonds(Nehra et al. 2012).

Chemical composition of the fresh and dried *P.ostreatus* and their active groups were studied by Gas Chromatography/ Mass Spectrometry (GC/MS), and FTIR analysis. The detected metabolites were classified as alcohols, alkanes, amides, esters, fatty acids, terpenoides and phenols(Mohamed and Farghaly,2014). *P.ostreatus* is rich in β-glucan, terpenoids and phenols which are responsible for the antimicrobial, antioxidant, antitumor and antiaging potentialities(Lindequist et al.2005). These bioactive compounds stimulate interleukin-12 production, nitric oxide synthase activation, free radical scavenging activity(Acharya et al.2005; Cui et al. 2005).

Estimation of the antioxidant activity, protein content and total phenols of mushroom in comparison with carrot:

Table 3: Antioxidant, protein, total phenols in *P.ostreatus* in comparison with carrot standard

Source	Protein content (µg/ml)	Antioxidant activity (%)	Total phenols (µg/ml)
P. ostreatus	475	27.8	302
***Daucus carota* (Egyptian Carrot)**	345.6	15.6	201

Protein content: As shown in table 3, Mushroom has higher protein content than that in the infusion of carrot (used as standard). It could be concluded that mushroom can be used as a safe dietary supplement. Mushroom is refined as a powder and is consumed in a capsules or tablets form. Its regular intake may enhance the immune response of human body.

The phenol content: Phenol content in *P.ostreatus* extract was significantly higher than in carrot infusion(Table 3).

Antioxidant activity: Table3 further revealed that mushroom showed higher potentiality in its scavenging activity than carrot so we concluded that it can be used as anti-tumor drug because the free radicals that arise in human body start to snatch and attack other cells to satisfy themselves causing cuts and mutations in the DNA sequences of the cell causing cancer.

In this connection, *P.ostreatus* have the highest nutritional value depend upon the presence of high protein content (40%)with high level of essential amino acids (arginine, alanine, glutamine and aspartic acid), carbohydrate(glucans, mannitol and trehalose), vitamins B, C, D, K(Çalarirmak et al.2007). Total phenol content and the antioxidant activity in green tea are significantly higher than that in *P.ostreatus*(Iwalokuu et al.2007). However, Mushroom phenolic compound has been found to be an excellent antioxidant and synergist that prevent the oxidative damage related to aging, diabetes and cancer measured by Psoriasis Area Severity index (PASI)(Cabrera et al., 2015). Antioxidant activities in *P.ostreatus* were found in metabolites includes, phenolic compounds, especially flavonoids and organic acids(Parikh et al.2005; Papaspyridi et al.2012). *P.ostreatus* membrane and cell wall are rich by selenium(Papaspyridi et al.2012) which act as antioxidant reduce the risk of cancer disease and enhance immune system(Sieja and Talercszyk, 2004; Papaspyridi et al. 2012). Hence the species of *Pleurotus* genus are considered to be an important source of dietary fiber, phenolic compounds & flavonoids which responsible for the antioxidant effect and the capacity to inhibit free radicals.

CONCLUSION

Mycelial extract of *P. ostreatus* is highly active against bacterial pathogens but not fungal pathogens compared with commercial antibiotics. Macro-dilution method is more sensitive than Agar-diffusion method in MIC determination. Tenfold dilution is more accurate than Bifold dilution. The combination therapy has more advantages than that of the monotherapy. Three synergistic mixtures were explored in this study and could be applied in the medicinal fields.

The results obtained from the protein content, total phenols and antioxidant activity in this study showed a high potentiality of mycelial extract from *P. ostreatus* as a potent therapeutic agent and food supplement.

REFERENCES

[1] Akyuz M, Kirbag S, (2009). Antimicrobial activity of Pleurotus eryngii var. ferulae grown on various agrowastes. Eur. Asia *J. Bio. Sci* 3, 8, 58-63.

[2] Akyuz M, Onganer AN, Erecevit P, Kirbag S. (2010). Antimicrobial activity of some Edible mushrooms in Eastern and Southeast Antatolia Region of Turkey. GU.J.Sci. 23(2):125-130.

[3] Alade PI, Irobi ON (1993). Antimicrobial activities of crude extract of Acalypha wilkesiana. J. Erthnopharmacol. 39: 171-174.

[4] Aqil F, Ahmad I, Owais M, (2006). Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. *Biotechnol. J.*1: 1093-1102.

[5] Aruna P, Fred R, Eugene M. (2001). Antioxidant activity. Medallion Laboratories Analytical Progress, 19 (1), 1-4.

[6] Bauer AW, Kirby WM, Sherris JC, Truck, M. (1966). Antibiotic susceptibility testing by a standar single dose method. *Am. J. Clin Pathol*. 45: 493-496.

[7] Benedict RG, Brady LR.(1972).Antimicrobial activity of mushroom metabolites.*J.Pharmaceut Sci*. 61(11):1820-1822.

[8] Bennett JE, Dismukes WE, Duma, RJ, Medoff G, Sande, MA, Gallis H, Leonard J, Fields BT, Bradshaw M, Haywood H, McGee ZA, Cate TR, Cobbs, CG, Warner JF, Alling DW.(1979). A comparison of amphotericin B alone and combined with flucytosine in the treatment of *Cryptococcal meningitis*. *N. Engl. J. Med.* 19:126-131.

[9] Cabrera S, Chinniah N, Lock N, Cains GD, Woods J. (2015). Inter-observer reliability of the PASI in a clinical setting. *Australas J Dermatol*.

[10] Çalarirmak N. (2007). The nutrients of exotic mushrooms Lentinula edodes and Pleurotus species and an estimated approach to the volatile compounds. Food Chem; 105: 1188-94.

[11] Cohen R., Persky L., Hadar Y.(2002). Biotechnological application and potential of wood-degrading mushrooms of the genus Pleurotus. Appl. *Microbiol. Biotechnol.* 58:582-594.

[12] Collins, R.A., Ng, T.B.(1997). Polysaccharopeptide from Coriolus versicolor has potential for use against human immunodeficiency virus type I infection.60(25):383-387.

[13] Conchran, KWST, Chang WA, Hayes(eds).(1978). Medicinal effects, In: The Biology and Cultivation of Edible Mushroom. Academic Press, New York, 160-187.

[14] Cordell, GA.(2000). Biodiversity and drug discovery symbiotic relationship. *Phytochemistry*. 55:463-480.

[15] Cui Y, Kim DS, Park KC (2005). Antioxidant effect of Inonotus obliquus. *J. Ethnopharmacol*. 96: 79 -85.

[16] Gao Y, Tang W, Gao H, Chan E, Lan J, Li X, Zhou S.(2005). Antimicrobial activity of the medicinal mushroom Genoderma. Food Rev.Int. 21:211-229.

[17] Ghannoum, MA, Elewski B.(1999). Successful treatment of fluconazole-fesistant oropharyngeal candidiasis by a combination of fluconazole and terbinafine. *Clin. Diagn. Lab. Immunol*. 6:921-923.

[18] Ghannoum, MA, Fu Y, Ibrahim AS, Mortara LA, Shafiq MC, Edwards JEJ, Criddle RS.(1995). *In Vitro* determination of optimal antifungal combination against Cryptococcus neoformans and Candida albicans. Antimicrob. Agents Chemother.39:2459-2465.

[19] Ghannoum, MA, Motawy MS, Abu Hatab MA, Ibrahim AS, Criddle RS.(1989). Multifactorial analysis of effects of interaction among antifungal and antineoplastic drugs on inhibition of Candida albicans growth. *Antimicrob. Agents Chemother*. 33:717-725.

[20] Iwalokum BA, Usen UA, Otunba AA, Olukoya DK. (2007). Comparativephytochemical evaluation, antimicrobial and antioxidant properties of*Pleurotus ostreatus*. Afr *J Biotech* 6: 1732-1739.

[21] Karacsonyi S, Kuniak L.(1994). Polysaccharides of *Pleurotus ostreatus*: Isolation and structure of pleuran, an alkali-insoluble Beta-D Glucan. Carbohyd. Polymer. 24:107-111.

[22] Kirkpatrick WR, Perea S, Coco BJ, Patterson TF.(2002). Efficacy of caspofungin alone and in combination with voriconazole in a guinea pig model of invasive aspergillosis. *Antimicrob. Agents. Chemother.* 46:2564-2568.

[23] Lee KI, Kim YJ, Lee HJ, Lee CY. (2001). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J. Agric. Food. Chem*, 51: 7292-7295.

[24] Lewis RE, Prince RA, Chi J,Kontoyiannis DP.(2002). Itraconazole preexposure attenuates the the efficacy of subsequent amphotericin B therapy in a murine model of acute invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. 46:3208-3214.

[25] Lindequist U, Niedermeyer THJ, Julich W. (2005). The pharmacological potentials of mushrooms. eCAM, 2: 285–299.

[26] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with folin phenol reagent.*J. Biol. Chem*.193, 265-275.

[27] Mencacci A, Cenci E, Bacci A, Bistoni F, Romani L.(2000).Host immune reactivity determines the efficacy of combination immunotherapy and antifungal chemotherapy in candidiasis. *J. Infect*. Dis. 181:686-694.

[28] Miguel JBG, Mirna EE, Tetsuya O. (1997). Volatile compounds secreted by the oyster mushroom Pleurotus ostreatus and their antibacterial activities. *J Agric Food Chem*; 45: 409. DOI:10.1021/jf960876i.

[29] Mohamed, EM, Farghaly, FA.(2014). Bioactive Compounds of Fresh and Dried Pleurotus ostreatus Mushroom. International Journal of Biotechnology for Wellness Industries, 3, 4-14.

[30] Moosavy MH, Shavisi N.(2013). Determination of Antimicrobial effects of Nisin and Mentha spicata essential Oil against *Escherichia coli* O157:H7 under various conditions (pH, Temperature and NaCl Concentration). *Pharmaceutical sciences.* 19(2):61-67.

[31] Mukherjee PK, Sheehan DJ, Hitchcock CA, Ghannoum MA.(2005). Combination treatment of invasive fungal infections. *Clinical Microbiology Reviews*. 163-194.

[32] Nair R, Chanda SV.(2007). Antibacterial activities of some medicinal plants of Westren Region of India. Turk. *J. Biol.* 31:231-236.

[33] National Committee for Clinical Laboratory Standards Performance standards for antimicrobial disk susceptibility tests (8th edn.), NCCLS, Wayne, PA (2003) Approved standard M2-A8.

[34] Nehra K, Meenakshi, Mukesh K, Ajay Y.(2012). Evaluation of antimicrobial potential of fruiting body extracts of Pleurotus ostreatus (oyster mushroom). International *J. Microbial Resource Technol*., Vol.1, No. 4, pp. 391-400. ISSN 2278 – 3822.

[35] Papaspyridi LM, Aligiannis N, Topakas E, Christakopoulos P, Skaltsounis AL, Fokialakis N. (2012). Submerged fermentation of the edible mushroom *Pleurotus ostreatus* in a batch stirred tank bioreactor as a promising alternative for the effective production of bioactive metabolites. Molecules; 17: 2714-24.

[36] Parihar S, Virani KD, Pithawala EA, Shukla MD, Lahiri SK, Jain NK, Modi HA.(2015). Phytochemical screening, Total phenolic content, Antibacterial and Antioxidant activity of wild Edible mushroom Pleurotus ostreatus. Int. Res. *J. Pharm.* 6(1).

[37] Parikh P, McDaniel MC, Ashen D, Miller JI, Sorrentino M, Chan V, Blumenthal RS, Sperling LS. (2005) Diets and cardiovascular disease an evidence-based assessment. J Am College Cardiol; 45: 1379-87.

[38] Pauliuc I, Dorica B.(2013). Antibacterial activity of Pleurotus ostreatus gemmotherapic extract. JOURNAL of Horticulture, *Forestry and Biotechnology*. 17(1):242-245.

[39] Sagakami H, Aohi T, Simpson A, Tanuma S.(1991). Induction of immunopotentiation activity by a protein-bound polysaccharide PSK. Anticancer Res. 11:993-1000.

[40] Sieja K, Talercszyk M.(2004) Selenium as element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol Oncol; 96: 559-61.

[41] Smania EFA, Delle Monache F, Smania A, Smania, A, Yunes, RA, Cuneo, RS.(2013). Antifungal activity of sterols and triterpenes isolated from *Ganoderma annulare*. Fitoterapia. 74(4):375-377.

[42] Suzuki H, Iiyama K, Yoshida O, Yamazaki S, Yamamoto N, Toda S.(1990). Structural characterization of immunoactive and antiviral water-solubilized lignin in an extract of the culture medium of Lentinus edodes mycelia (LEM). *Agric. Biol. Chem*. 54(2):479-487.

[43] Tajkarimi M, Ibrahim SA.(2011). Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on *Escherichia coli* O157:H7 in laboratory medium and carrot juice*. Food Control*. 22(6):801-4.

[44] Vamanu E. (2012). Biological activities of the polysaccharidesproduced in submerged culture of two edible *Pleurotus ostreatus*mushrooms. *Journal of Biomedicine and Biotechnology*. 2012: 1-8.

[45] Vani T, Rajani M, Sarkar S, ShishooCJ. (1997). Antioxidant properties of theayurvedic formulation Triphala and itsconstituents, Inter. *J. Pharmacognosy*, 35, 313-317.

[46] Wang, H, Ng, TB.(2004). Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii*. Peptides. 25:1-5.

[47] Wasser SP, Weis, AL.(1999). Medicinal properties of substances occurring in higher Basidiomycetes muhrooms: Current perspectives (Review). Int. J. Med. Mushr. 1:31-62.