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Therapeutic properties of oyster mushroom (*P. ostreatus*) cultivation on different cellulosic substrates

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ABSTRACT: The objectives of this work were to evaluate the antimicrobial activities, antioxidant in vitro cytotoxicity and DNA damage of *Pleurotus ostreatus* (Jacq.) P. Kumm. cultivation on some local cellulosic substrates. It observed to be very active against bacteria (except *S. mutans* and *K. pneumonia*), yeast and dermatophytes when compared to the control groups (10.0-32.7 mm). It also showed significant antioxidant potential, the highest TAS (0.978 mmol/L) and TOS (10.51 µmol/L) values were obtained in M-P (1:1) and W, and also DPPH radical scavenging effects was more effective at 25 mg (74.80%) on MS. The methanol extract of *P. ostreatus* cultured on M-W (2.35%) and M-P (1:1) (9.74%) at 400 µg/mL concentration has remarkably decreased the percentage of viability in MDA-MB-231 and A-549 cell lines. However, it has been determined that *P. ostreatus* does not have a DNA protective effect. These results indicate that *P. ostreatus* has potent antimicrobial, antioxidant, as well as cytotoxic effects.

KEYWORDS: *P. ostreatus*; edible mushroom; medicinal effect; cytotoxicity; DNA damage

1. INTRODUCTION

Reactive oxygen species such as hydrogen peroxide, superoxide anion and hydroxyl radical are produced as byproducts of biological reactions or exogenous factors. They are constantly produced during metabolism and damage biomolecules and cause serious health problems associated with aging, cancer, rheumatoid arthritis and atherosclerosis [1]. It is also known that they cause food spoilage by inducing lipid peroxidation [2], cause membrane fluidity and damage by inducing oxidation of lipids and DNA, and also cancer-causing changes through DNA mutation [3]. Macrofungi have bioactive compounds with functional and medicinal characteristics. They are a natural source of antioxidants and help prevent oxidative stress when included in the diet [4].

The genus *Pleurotus* belonging to the Pleurotaceae are well commonly known as Oyster mushrooms, and used for their gastronomic properties, medicinal characteristics, and their ability to grow on different substrates. They have always been valued and appreciated for their high nutritional content, distinctive taste and texture, unique taste, and enormous role in curing various degenerative diseases such as immunomodulatory, antioxidant, antiinflammatory, hypocholesterolemic, antigenotoxic, antihyperglycemic, antiviral, anti-HIV, antitumor, antimutagenic, hepatoprotective, antiaging and antiallergic effects etc. [5-8]. *P. ostreatus* is the second largest commercially grown mushroom worlwide due to its cheaper production technology and simple, high biological productivity, wide climatic conditions, little environmental control, shorter growing periods, its fruit bodies are rarely affected by pest and diseases compared to other [9].

Nowadays, when scientific studies are examined, it is seen that studies on medicinal plants are common. It is known that plants are mostly used for therapeutic purposes, to obtain medicine, to treat various diseases and to help treatment. Turkey is rich in medicinal plant diversity as well as mushroom diversity. Especially in Far East countries, medicinal mushrooms have been used for centuries both as a source of food and in the treatment of various diseases. In recent years, it has been shown that many bioactive components detected in mushrooms can be used in the treatment of various diseases. Most of the research has focused on the various medicinal properties including cytotoxic, DNA protective activity, antioxidant and antimicrobial effects of edible macrofungi [3-8] that have become popular in recent years. Turkish people have a tradition of using various types of mushrooms for food instead of using them in the treatment of infectious diseases and various ailments. For this reason, before using *P. ostreatus*, a delicious

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mushroom that grows in nature and has a market share in our country, it is necessary to know whether it has different medicinal effects. Therefore, this study was conducted to evaluate the antimicrobial activities, antioxidant cytotoxicity and DNA protective effect of *P. ostreatus* cultured on local cellulosic substrates.

2. RESULTS AND DISCUSSION

Antimicrobial effects of *P. ostreatus* cultured on M-W (1:1), M-P (1:1), W and M indicated activity on other test microorganisms at different ratios (see Table 1). The methyl alcohol extract of *P. ostreatus* was observed to be changeable statisticaly against bacteria, yeast and dermatophte when compared with the control group (see Table 1, $p<0.05$). While the methanol extract of *P. ostreatus* present lower activity against *S. mutans* (18.0-28.3 mm) and *K. pneumoniae* (17.0-23.7 mm), it showed high activity against all other bacteria, yeast and dermatophytes (17.7-30.0 mm) compared to the control group (see Table 1, $p<0.05$). In addition, minimal inhibitor concentration (MIC) may vary for the studied species (31.25-62.5 µg/mL) shown Table 1. Methanol extract of *P. flabellatus* has antimicrobial effect against test organisms with average MICs ranging from 26.03 to 83.3 mg/mL. *P. mirabilis* had the lowest mean MIC (26.03 mg/mL) antimicrobial activity, while *S. aureus* had the highest mean MIC with 83.3 mg/mL [10]. Many investigators reported that extracts from the fruiting body of *Pleurotus* species exhibit antibacterial activity against different test microorganisms. Younis et al. [11] indicated that the water extract of *P. ostreatus* had the strongest effect in inhibiting the growth of most fungi. The test microfungi most susceptible to inhibition were *C. albicans*, *C. humicola*, and *T. cutaneum*, and the most susceptible test bacteria were *S. aureus* followed by *E. coli*, although alcohol-based solvents in all samples had less antimicrobial activity against most tests microorganisms. Gashaw et al. [12] showed the highest antibacterial activity of *P. ostreatus* and *P. florida* against *E. coli* (19.8 mm) and *P. aeruginosa* (16.4 mm), and *E. coli* (18.6 mm) and *S. faecalis* (14.8 mm), respectively.

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Table 1. Antimicrobial activities (mm) and MIC test (µg/mL) of *P. ostreatus* cultured on various local cellulosic wastes

Compost Medium (1:1)	Bacteria												Yeast		Dermatophytes			
	Gram (+)				Gram (-)													
	S. mutans		*S. aureus*		*P. vulgaris*		*P. aeroginosa*		*K. pneumoniae*		*E. coli*		*C. tropicalis*		*Epidermophyton* sp.		*Trichophyton* sp.	
		MIC		MIC		MIC		MIC		MIC		MIC		MIC		MIC		MIC
W	22.3±0.6^{b}	-	28.0±0.0^{c}	31.25	23.7±0.6^{d}	-	21.3±0.6^{c}	31.25	17.3±0.6^{a}	31.25	27.7±0.6^{d}	31.25	25.7±0.6^{b}	-	23.7±0.6^{c}	31.25	17.0±0.0^{d}	31.25
M	28.3±0.6^{c}	-	29.0±0.0^{d}	31.25	24.0±0.0^{d}	-	21.7±0.6^{c}	31.25	23.7±0.6^{c}	62.50	30.0±0.0^{e}	31.25	20.3±0.6^{a}	-	28.7±0.6^{d}	31.25	28.0±0.0^{e}	31.25
M-W	18.0±0.0^{a}	-	29.3±0.6^{d}	31.25	19.3±0.6^{c}	31.25	19.7±0.6^{b}	31.25	17.0±0.6^{a}	31.25	25.7±0.6^{b}	62.50	27.7±0.6^{c}	-	20.3±0.6^{b}	62.50	13.3±0.6^{b}	62.50
M-P	21.7±0.6^{b}	-	24.0±0.0^{b}	62.50	17.7±0.6^{b}	31.25	20.7±0.6^{bc}	62.50	19.7±0.6^{b}	62.50	26.7±0.6^{c}	31.25	25.3±0.6^{b}	-	20.0±0.0^{b}	62.50	15.7±0.6^{c}	62.50
Control	**32.7±0.6^{d}**		**14.7±0.6^{a}**		**11.3±0.6^{a}**		**10.3±0.6^{a}**		**18.0±0.6^{a}**		**10.0±0.0^{a}**		**29.3±1.2^{d}**		**14.7±0.6^{a}**		**11.7±0.6^{a}**	
F value	383.375		852.500		301.625		203.700		62.700		957.500		64.688		299.125		617.167	
***p* value**	0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000	

W: *Wheat straw*, M: *Medicago sativa* L., P: *Prangos pabularia* Lindl., MIC: Minimal inhibitor concentration
Test microorganism: *Staphylococcus aureus* COWAN 1, *Streptococcus mutans*, *Proteus vulgaris* FMC 1, *Pseudomonas aeruginosa* DMS 50071 SCOTTA, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Candida tropicalis* ATCC 13803, *Epidermophyton* sp., *Trichophyton* sp.
Comparison antibiotic were used for bacteria (Streptomycin sulfate (10 µg/disk)), yeast and dermatophyta (Nystatin 30 µg/disk)
Values are means of three replicates ±SD, Means in the same column with the different superscript are significantly different (p<0.05)

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Schillaci et al. [13], noted that the extracts of *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. eryngii* var. *elaeoselini*, and *P. nebrodensis* analyzed inhibited the tested microorganisms (*S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*) in varying degrees. It seems that the antimicrobial activity of *P. ostreatus* (see in Table 1) are changeable as reported by other above mentioned researchers. The antimicrobial effect of macrofungi extracts is highly dependent on the fungal species, growing conditions, method of preparation of the extracts, evaluation and analysis of the findings. They have various compounds in varying concentrations and this explains the disparity in antibacterial activity as demonstrated in the studies mentioned above.

The total antioxidant status (TAS), total oxidant status (TOS) ratio and 2.2-diphenyl-1-picrilhydrazyl (DPPH) activity of *P. ostreatus* cultivation on various local substrates were determined to be very changeable as seen in Table 2 ($p<0.05$). The highest TAS (0.978 mmol/L) and TOS (10.51 µmol/L) ratio were obtained on M-P (1:1) and W, but the lowest were on W (0.704 mmol/L) and M (4.46 µmol/L) (see Table 2). Based on the literature review, the TAS and TOS value content for mushrooms were determined as 2.3-3.1 mmol/L TAS and 1.2-10.8 µmol/L TOS for *P. citrinopileatus* cultivated on various substrates [14], 1.4-1.9 mmol/L TAS for *P. eryngii* obtained from wild sample [15], 1.4 mmol/L TAS and 6.6 µmol/L TOS for *P. eryngii* collected from natural sample [16], 3.5-4.4 mmol/L TAS and 6.6-12.3 µmol/L TOS for *P. djamor* grown on cellulosic residues [17], 1.2-2.0 mmol/L TAS and 1.1-7.0 µmol/L TOS for *P. ostreatus* obtained from culture and wild samples, and also 1.1-1.3 mmol/L TAS and 4.2-11.5 µmol/L TOS for *A. bisporus* obtained from wild and cultivated samples [18]. The highest TAS ratio of *P. ostreatus* cultivated on M-P (1:1) (0.978 µmol/L) was found to be lower than those reported in several studies [14-18], and also the highest TOS (10.51 µmol/L) ratio obtained from W were found to be similar to those reported in several studies [14], lower than those reported by others [17, 18], and higher than those reported by others [14, 16-18].

Table 2. TAS, TOS values and DPPH of *P. ostreatus* cultured on some local lignocellulosic wastes

Materials (1:1)	TAS (mmol/L)	TOS (µmol/L)	DPPH (%)			
			25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL
W	0.704	10.51	63.82	25.00	7.32	6.71
M	0.738	4.46	74.80	31.91	10.98	1.42
M-W	0.723	7.04	64.23	22.76	5.25	3.69
M-P	0.978	6.45	68.29	32.93	9.35	2.44

W: *Wheat straw*, M: *Medicago sativa* L., P: *Prangos pabularia* Lindl.
TAS: the total antioxidant status, TOS: total oxidant status, DPPH: 2.2-diphenyl-1-picrilhydrazyl

As seen in Table 2, the DPPH effect could be seen in every group, but this effect, which we evaluated as a percentage, increased in direct proportion to the increase in the amount of sample. When the groups were compared in terms of concentration, the highest DPPH level was seen in the MS medium with 74.80% in the group to which we added 25 mg sample (see Table 2). It has been determined by different researchers that mushroom extracts have antioxidant effects by using different methods (DPPH, ABTS, FRAP, ORAC, antiradical, reducing power, chelating ability etc.). Akyüz et al. [19] noted that DPPH ratios were found to be 22.97-91.89% at 50-800 µL for *Agaricus* spp., *Pleurotus* spp., *M. esculenta* and *T. boudieri*. Elhusseiny et al. [20] studied that the DPPH assay (IC_{50}) showed that the free radicle scavenging ability of *P. columbinus* (35.13 µg/mL) was significantly higher than *P. sajor-caju* (40.91 µg/mL) and much higher than that of *A. bisporus* (83.93 µg/mL). Adebayo et al. [4] investigated that the DPPH assay (µg/mL^{-1}) were recorded in *P. pulmonarius* (0.49-0.70 µg/mL^{-1}), *P. levis* (0.51 µg/mL^{-1}), *P. tuber-regium* (1.68l µg/mL^{-1}) and *P. ostreatus* (0.63-1.05 µg/mL^{-1}). Our data (see Table 2) differ from those reported by other investigators. Variability in DPPH activities of *P. ostreatus* extracts may be due to variations in bioactive formulations or concentrations, types of fungi, extraction methods, and modes of action of active substances, as demonstrated in the studies cited above.

As seen in Table 3, a variable cytotoxic effects were observed on MDA-MB-231 and A-549 cell lines at 200 µg/mL and 400 µg/mL concentrations of the methanol extract of *P. ostreatus* grown on W, M, M-W (1:1) and M-P (1:1). Methanol extracts of *P. ostreatus* were shown to be variable against cell lines A-549 and MDA-MB-231 when compared to negative (DEMEM) and positive (doxorubicin) control groups (see Table 3). The

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methanol extracts of *P. ostreatus* cultured on W, M, M-W (1:1) and M-P (1:1) show low cytotoxic activity on cell line MDA-MB-231 and A-549 at the concentrations of 200 µg/mL, but showed high cytotoxic activity on the same cell lines at 400 µg/mL, respectively (see Table 3). As a results, A-549 and MDA-MB-231 cells showed higher sensitivity to the methanol extract of *P. ostreatus* obtained from M-P (1:1) (9.74%), M (2.41%) and M-W (1:1) (2.35%) at 400 µg/mL concentration, respectively (see Table 3). It was determined that various solvents and bioactive components extracted from different mushroom species (*P. djamor*, *P. ostreatus*, *P. nebrodensis*, *P. eryngii*, *P. ferulae*, *P. tuber-regium*, *P. columbinus*, *P. sajor-caju*, *A. bisporus*, *P. pulmonarius*, *Terfezia* spp. and *Picoa* spp.) had dose-dependent cytotoxic effects on H1299, HUVEC, PC3, SiHa, HeLa, EAC, HepG2, BV2, HEK-293, MCF7, A549, BGC-823, HGC-27, MDA-MB-231, MRC-5, RAW 267.4, Colo-205, LS-513, Du-145, HT29, HCT-116, HCT-116, Lu-04, Bre-04, L929 cell lines [20-30]. Our data (see Table 3) may differ from those reported by the other investigators mentioned above. Large quantitative differences (possibly due to analytical methods used and fungal species) and heterogeneity of samples analyzed, cancer cell line tested, various assays were found to be so in the cited studies.

Table 3. Cytotoxic effect of *P. ostreatus* extracts on cell lines (%)

Concentration	Materials (1:1)	Cell Line	
		A-549	**MDA-MB-231**
200 µg/mL	**W**	91.38	29.64
	M	89.07	29.76
	M-W	75.25	56.93
	M-P	82.76	23.52
400 µg/mL	**W**	24.18	26.11
	M	11.19	2.41
	M-W	14.68	2.35
	M-P	9.74	7.06
Doxorubicin		**49.20**	**10.69**
Negative Control		**100.00**	**100.00**

W: *Wheat straw*, M: *Medicago sativa* L., P: *Prangos pabularia* Lindl.
Values are means of three replicates ±SD.
Doxorubicin was used as positive control and DEMEM as negative control.
A-549: Human lung cancer cell line, MDA-MB-231: human breast cancer cell line

It has been observed that each extract of *P. ostreatus* grown on different cellulosic residues has different therapeutic properties. It was observed that the difference in the culture medium had a significant effect on the different antibacterial, antioxidant and cytotoxic effects of the extracted extracts (see Tables 1, 2 and 3). The biological structure of different culture media has an effect on the nutrients and bioactive components of the mushroom, and will therefore create different effects on the medicinal effects (show Table 1, 2 and 3). Considering all this, it supports the findings of the previously mentioned researchers [11-30].

The DNA protective effects of MetOH extracts of *P. ostreatus* grown on W, M, M-W (1:1) and M-P (1:1) substrates were determined using the plasmid pBR322 DNA screening method in gel electrophoresis (see Figure 1). As seen in Figure 1, DNA preserved its stable structure in the wells with K1 and Oxibenzone. The supercoiled structure of DNA is seen in these wells. With the effect of UV and H_2O_2 in K2, DNA could not maintain its stable structure and a smear image was formed. It has been determined in the study that it does not have a DNA protective effect because DNA cannot preserve its stable structure in the 1st, 2nd, 3rd and 4th bands. According to our results, MetOH extract of *P. ostreatus* cultured on W, M, M-W (1:1) and M-P (1:1) substrates did not showed a protective effect to plasmid pBR322 DNA form damage caused by UV and H_2O_2 at a concentration of 25 mg/mL (Figure 1, Lane 1-4). Kim and Kim [31] stated that a water-soluble polysaccharide from *G. lucidum* is protective against DNA strand breaks caused by the hydroxyl radical, and Pillai et al. [32] suggested that the radioprotective properties of the aqueous extract of *G. lucidum* against radiation-induced plasmid pBR322 DNA strand breaks may be due to inhibition of lipid peroxidation. Zhao et al. [33] demonstrated potent DNA protective effects from oxidative damage reported for protein extracts from selenium-enriched *G. lucidum* (Se-GLPr). Zhao et al. [34] reported that polysaccharide extracts from Se-enriched *G. lucidum* protected DNA from hydroxyl radical oxidative damage in a dose-dependent manner. Akyüz et al. [35] noted that *T. olbiensis* and *P. lefebvrei* extracts showed a DNA protective effect. At a concentration of only 40 mg/mL, the water extract of *P. juniperi* showed a DNA protective effect, while the

T. boudieri did not show any DNA protective effect at all concentrations tested. As a result of the imbalance between oxidant and antioxidant defense mechanisms, many diseases occur. Edible mushrooms can protect DNA from damage caused by hydroxyl, superoxide anion, and hydrogen peroxide radicals, furthermore, they are defined by various oxidation process steps and different mechanisms, as noted in previous studies [31-35].

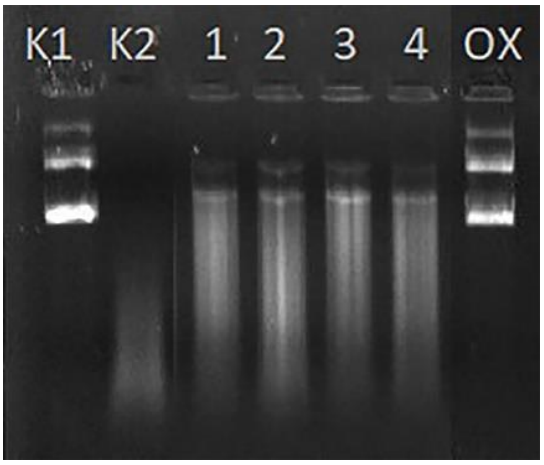

Figure 1. Electrophoretic pattern of pBR322 plasmid DNA after treatment with dH_2O, UV and H_2O_2 in the presence of MetOH extracts [K1: Plasmid DNA (3 µL) + dH_2O (6 µL), K2: Plasmid DNA (3 µL) + dH_2O (6 µL) + H_2O_2 (1 µL) + UV, 1: Plasmid DNA (3 µL) + 25 mg/mL of *P. ostreatus* (M-P) MetOH extracts + UV + H_2O_2 (1 µL), 2: Plasmid DNA (3 µL) + 25 mg/mL of *P. ostreatus* (M) MetOH extracts + UV + H_2O_2 (1 µL), 3: Plasmid DNA (3 µL) + 25 mg/mL of *P. ostreatus* (W) MetOH extracts + UV + H_2O_2 (1 µL), 4: Plasmid DNA (3 µL) + 25 mg/mL of *P. ostreatus* (M-W) MetOH extracts + UV + H_2O_2 (1 µL), OX: Plasmid DNA (3 µL) + Oxibenzon (5 µL) + UV + H_2O_2 (1 µL) respectively].

3. CONCLUSION

As a result, it was observed that *P. ostreatus* had strong antimicrobial, antioxidant and cytotoxic activities, but did not have a DNA protective effect. It was observed that the best DPPH, cytotoxic and antimicrobial effect values of *P. ostreatus* grown in various local cellulosic wastes were obtained from M medium, but TAS and TOS values were more effective in M-P (1:1) and W medium, respectively. In addition, considering the richness of the medicinal contents of *P. ostreatus*, the use of M wastes in the culture medium will be beneficial.

4. MATERIALS AND METHODS

4.1. Obtained of mushroom samples

The mushroom samples used in the present research were obtained from previous cultural studies [36]. The sample was shade dried for 10 days, pulverized and stored till ready for use.

4.2. Analytical methods

Selected medicinal activites of *P. ostreatus* cultured on various local lignocellulosic residues including antimicrobial effect and MIC value, total antioxidant and oxidant activity, DPPH, cytotoxicity and DNA damage were determined with appropriate methods, as described below: Antimicrobial effect of the methanol extract of P. ostreatus were determined according to the disk diffusion method [37]. Minimal inhibitor concentration tests were conducted on the mushroom MetOH extracts using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The lowest concentration that prevented the proliferation of bacteria, yeast and dermatophyte fungi were determined as the minimal inhibitor concentration (MIC) [38]. Total antioxidant status (TAS) and total oxidant status (TOS) of mushroom extracts were analyzed with Rel Assay kits by modified by Erel [39, 40]. The antioxidant activity was carried out by the 2.2-diphenyl-1-picrilhydrazyl (DPPH) (the absorbances of each mixture were read at 570 nm in the elisa reader) radical scavenging capacity method [41, 42]. The anticancer activities of *P. ostreatus* on MDA-MB-231 and A-549 cell lines were determined by using 3-(4,5-dimethylthiazol–2-iyl)-2,5-diphenyl tetrazolium bromide (MTT) assay method [43]. The DNA protective effect was evaluated using plasmid pBR322 DNA

treated with UV and H_2O_2 treatment in the presence of MetOH extracts and checked on 1.25% agarose gels according to literature [44, 45] after some modifications.

Author contributions	**:** The authors declare that they have contributed equally to the article.
Conflict of interest statement	**:** The authors declare no competing interests.
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Ethics Approval	**:** No ethical approval required.

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