Original scientific paper
UDC 582.284.51:547.917]:615.282(567)

EVALUATION OF ANTIFUNGAL POTENTIAL OF D-B-GLUCAN EXTRACTED FROM THE FRUITING BODIES OF *PLEUROTUS OSTREATUS*

Safaa Mahmmod Sultan[1,2*], Faten Noori MulaAbed[1]

[1]Department of Biology Sciences, College of Science, University of Mosul, University street 94GW+J9, 41002 Mosul, Iraq
[2]Department of Medical Laboratory Techniques, Mosul Technical Institute, Northern Technical University, University Hwy street 95H3+JM, 41002 Mosul, Iraq

[*]e-mail: sultansafaa85@gmail.com

Abstract

The recent development of fungus-resistant strains has caused concern in healthcare settings in Iraq. This has led to increased research interest in Basidiomycetes because it contains compounds with high medical efficacy. The current research explores the medical potential of the compound D-β-glucan extracted from the fungus *Pleurotus ostreatus* as an antifungal agent against clinical fungal pathogens that are resistant to the current generation of antifungals.

During this study, samples of a fungus belonging to the genus *P. ostreatus* were obtained from different locations in the city of Mosul - Iraq. Standard isolations of *Candida* sp. species include *Candida albicans, C. glabrata, C. tropicales,* and *C. lusitaniae* which were obtained and activated. The active compound D-β-glucan was extracted using the Soxhlet extractor and purified from *P. ostreatus* using high-performance liquid chromatography (HPLC) and for the aqueous and ether extractors. The antifungal effect of D-β-Glucan complex was tested using enzyme-linked immunosorbent assay (ELISA), and turbidity or growth density was observed based on optical density (OD).

The results showed that the highest concentration of D-β-glucan aqueous extract was 9.842 µg/mL in Mosul forest area, followed by 3.171 µg/mL for the Alhadba' farms, while the highest concentration of D-β-glucan in the ether extract was 265.205 µg/mL for the Mosul forest area, followed by the Alsalamia, where the concentration was 143.368 µg/mL. Finally, the highest inhibitory concentration against *Candida lusitaniae* was found at 200 pg/mL at OD 0.086, while the lowest OD value for *Candida gabrata* was 0.143 at the same concentration.

We conclude through the study that it is possible to extract D-β-glucan compound from Basidiomycetes, including *P. ostreatus*, and we can conclude that D-β-glucan has the ability to inhibit the growth of fungi.

***Key words**: Basidiomycetes, Pleurotus ostreatus, D-β-glucan, Antifungal, Candida sp.*

1. Introduction

Until the end of the twentieth century, researchers were interested in the fungi ability (especially Basidiomycets fungi) to produce effective medicinal compounds, including antibiotics [1]. Fungi are the most diverse ecosystem group of organisms, and they are very important at a time when the world is facing difficult times in environmental management. Within the framework of efforts to eliminate many environmental problems, real reflection is to search for them in all parts of the world. Reasons for this are multiple. For example, the filament fungal for variety of reasons are responding by firing chemical signals in all directions, similar to the nervous system to a large extent, which can be used to manage the Earth's ecosystem [2]. These organisms spend half of their lives invisible and underground, rupturing the tissues

of dead plants and spreading in the form of a pearly white spider web without a specific shape and in large numbers that may reach five times the number of plants. Researchers have wide scope to discover and determine thousands of species that are still unknown, that certainly exist among us [3]. This kind of research is neglected in Iraq, and this is sad at a time when the whole world is moving to use the natural products of fungi in various manufacturing processes in the same time trying to ensure its food safety and health of consumers [4].

Figure 1. *Pleurotus ostreatus*

One of the mushrooms that belongs to the phylum Basidiomycota and its produced and used as food fungi is *Pleurotus ostreatus*, (Figure 1), called oyster mushrooms. It is growing in the range of 12 - 32 ^{0}C [5]. This mushroom is characterized by its relatively simple cultivation, because it grows in a variety of cellulose waste that is utilized due to its enzymatic compounds. In the same time, this mushroom has great nutritional value because it is an excellent source of proteins, carbohydrates, vitamins and minerals, and it contains low fat and cholesterol-free matters [6]. It is the third most common species in the world, after the fungus *Agaricus bisporus*, and the *Shiitake* spp. [7]. Poland is the main producer of oyster mushrooms in Europe with annual production exceeding 80,000 tons [8]. *Pleurotus ostreatus* is one of the most common and most studied species. It belongs to phylum Basidiomycota, order Agaricales, family Pleurotaceas. Most often, solid-state cultivation is used for fruiting body formation by *P. ostreatus* using lignocellulosic side-streams from the agro-industrial production of food as a substrate [9]. The fruiting body formation of *P. ostreatus* could take from 20 to 25 days. Submerged cultivation has recently received increasing attention, because it could lead to the efficient production of mycelia with uniform and reproducible qualities and valuable metabolites in a much shorter time [10]. The fruiting bodies of *P. ostreatus* have excellent organoleptic properties and high nutritional value, and mankind has consumed it's for thousands of years. The metabolites derived from basidiocarps and mycelia demonstrated enviable medicinal properties [11]. These mushroom species are a significant source of a variety of bioactive compounds, such as: polysaccharides, soluble and insoluble glucans, dietary fibers, proteins, polyphenols, and macro- and microelements. One of the most studied polysaccharides is the water-insoluble (1-3)-β-d-glucan called "pleuran", which is obtained from *P. ostreatus* [12]. The above-mentioned bioactive substances demonstrated many medicinal activities including antibacterial, hypoglycemic and antihyperlipidemic properties [13]. Mushrooms' metabolites can also control the immune system, suppress tumor growth and inflammation, lower blood cholesterol levels, prevent high blood pressure and atherosclerosis, and perform many other functions [14].

The recent development of fungus-resistant strains has caused concern in healthcare settings in Iraq. This has led to increased research interest in Basidiomycetes because it contains compounds with high medical efficacy. The current research explores the medical potential of the compound D-β-glucan extracted from the fungus *Pleurotus ostreatus* as an antifungal agent against clinical fungal pathogens that are resistant to the current generation of antifungals.

2. Materials and Methods

2.1 Collection of samples

Search of the fruiting bodies of the fungus *Pleurotus* spp., from different sites of Mosul was done in the period from 7 November 2021 to 21 January 2022, in an area covering 10,000 m^2. These sites were selected because they constitute a rich environment for fungal diversity due to the large number of fallen leaves that covered its floor. The fruiting bodies were collected from their natural habitats (trunks and cavities of the live, dead and cut trees, and bushes) using hands and scalpels. They were kept in plastic bags on which the information was fixed (collection site, tree type and description, and date of collection).

2.2 Primary determination

The initial determination was carried out by inoculating potato dextrose broth with five discs of each isolated fungal colony for 7 days. Then they were fortified in the vibrating incubator for 7 days at a temperature of 25 ± 2 ^{0}C with a shaking speed of 150 cycles/minute.

2.3 Phenotypic determination of selected, isolated fungi

The isolated *P. ostreaus* were determined depending on: body shape, fruiting, and color. Parts of the fruiting body were immersed in 70% ethanol, and tissue was taken from inside the fruiting body and under sterile conditions cultured on the potato dextrose agar (PDA)

medium prepared previously. Incubation was done at a temperature of 25 ^{0}C for 4 days. After that we made sub-culturing on the same medium with incubation at the same temperature for 7 days. Texture, as well as the vegetative and sexual components of these isolated fungi were examined using methylene blue dye at a magnification of 40X, and by light microscope at a magnification of 100X without dye.

The samples were transferred to the laboratory after the end of each trip for harvesting *P. ostreaus* fruiting bodies. Then they were cleaned from the dust and impurities, and washed with running water for several times. After that they were immersed in 70% ethanol for five minutes. The pieces of the fruiting bodies were left to dry on sterile filter papers Whatman No.1 and inoculated at the rate of 1 - 2 pieces on PDA medium at a temperature of 28 ± 2 ^{0}C for 7 - 14 days. The cultures were coated with aluminum foil until the appearance of fungal colonies [15].

2.4 Extraction of active components from the extract

Extraction was done by Soxhlet. This is a method used in chemistry used for extraction of compounds from solids or liquids. It is a more complex process compared to simple hydrolysis, but it can be more effective in extracting specific compounds, including active ingredients in sample.

2.5 HPLC technique

2.5.1 Identification of active ingredient

The active ingredient in the extracts was identified by comparing the retention time and UV spectra with those of the standard compounds. The standard compound used was showed in Table 1 below. Basidiomycetes extract used in this study is *Pleurotus* spp. extract.

Table 1. The standard compound was used under study

No.	Compounds	Concentration
1	β-D-glucan (pluran)	40)µg/mL/mL(
2	Hexandichloromethane	1 (g/mL)
3	Tannin	1 (g/mL)

2.5.2 Extraction procedure

Two different extraction methods were employed: petroleum ether extraction and water extraction. For the petroleum ether extraction, 25 g of dried and powdered *Pleurotus* spp. was mixed with 400 mL of petroleum ether and refluxed for 72 hour. The extract was then filtered through filter paper and concentrated using a rotary evaporator. The water extraction was carried out by boiling 25 g of dried and powdered *Pleurotus* spp. with 100 mL of distilled water for 72 hour. The extract was then filtered and freeze-dried to obtain a powder [16].

2.5.3 Preparation of HPLC samples

The extracts obtained from the two extraction methods were dissolved in water and 10 mg were taken in order to prepare the HPLC samples. The samples were then filtered through a 0.22 µm filter before being injected into the HPLC system [17].

2.5.4 HPLC analysis

HPLC analysis was carried out using Shimadzu, model 2022. The column used was C18. The mobile phase consisted of methanol : water at 80:20 % for tannin , water : acetonitrile at 25 : 75, and water for β-D-Glucan. The flow rate was set to 1mL/min. The injection volume was 20 µL/mL. The detection wavelength was set at: 230, 270 and 410 for hexane dichloromethane, tannin, and β-D-Glucan respectively. The HPLC chromatograms were analyzed using Shimadz model 2022 software [18].

2.5.5 Identification of active ingredient

The active ingredient in the extracts was identified by comparing the retention time and UV spectra with those of the standard compound. The standard compound used were β-D-glucan, tannin and hexane dichloromethane 100% [19].

2.5.6 Determination of extract yield

The extract yield was determined by dividing the weight of the extract obtained by the weight of the plant material used for the extraction [20].

2.5.7 Determination of purity

The purity of the active ingredient in the extracts was determined by calculating the peak area of the active ingredient and comparing it with the total peak area of all the compounds in the extract. The purity was expressed as a percentage [21].

2.6 Effect of β-D Glucan (pluran), against some types of *Candida spp.*

The microplate method was adopted to determine the antagonistic activity of the using the half-dilution method against some types of yeasts according to the method of determining the minimum inhibitory concentration (MIC) according to the test protocol Microplate MIC Assay, described by the Clinical and Laboratory Standards Institute [22].

3. Results and Discussion

3.1 Macroscopic determination

After collecting the samples, they were washed with running water, and the initial diagnosis of selected isolates of the fungus was made based on macroscopic phenotypic characteristics. It has been observed to be a species of edible wild fungus. The oyster mushroom is so named because of its oyster-like head shape in diameter from 5 to 25 centimeters approximately, and is characterized by its white, golden, or grey color gradient between light and dark. Its surface is smooth with wavy edges. It can be found on the trunks of tree species in a saprophytic groups, with flesh that is white, firm, and varies in thickness due to the layers arrangement. The gills of the mushroom are white to cream. The spore print of the mushroom is white to lilac-gray, and it is best viewed on dark background.

As per the finding of this study, the morphological characteristics of *Pleurtous ostreatus* were more or less similar with Sultan *et al.*, [23], who reported that the pileus of *Pleurotus* spp. was soft, smooth, light yellow with diameter of 4.5 - 11 cm. It possessed white, broad decurrent gills with often lateral stipe, usually elongated from 5 to 8 cm, with white, solid, several fruit bodies joined at the base to form a large common base. The fresh sample was soft, white with mild odour [23], showed that *Pleurotus* sp. was morphologically characterized by white spores with an eccentric or lateral stem of fleshy texture. Cap was 2 - 15 cm wide and 3 - 11 cm long, and upper surface was smooth with white, spathulate to kidney shaped, with a margin that was decurved or inrolled. Stem was usually short or stem poke base, imbricated in groups of 5 - 20 cm. Gills were 18 - 20 cm at margin, 5-15 mm wide, decurrent, sometimes uniting to form a net or pore like pattern on the stem, white when fresh, yellowish when dried [24]. Isolated oyster mushrooms from forest region, based on microscopic view and phylogenetic tree, were identified as *Pleurotus* spp. [25] (Figure 2).

3.2 Microscopy determination

In order the basidia and basidospores to be determined, the mycelium was examined under the microscope at and 40X using methylene blue dye, but also with magnification of 100X without dye (Figure 2).

Spores dimensions were: 7 - 11 x 2 - 4 µm, and by form they were cylindrical to ellipsoid and smooth. Hymenial cystidia were not found. Pileipellis was partially gelatinized, with tangled cutis of elements, 2.5 - 10 µm wide, smooth, hyaline to yellowish in KOH, inconspicuously clamped, as in the work of Kimic *et al.*, [26] (Figure 3).

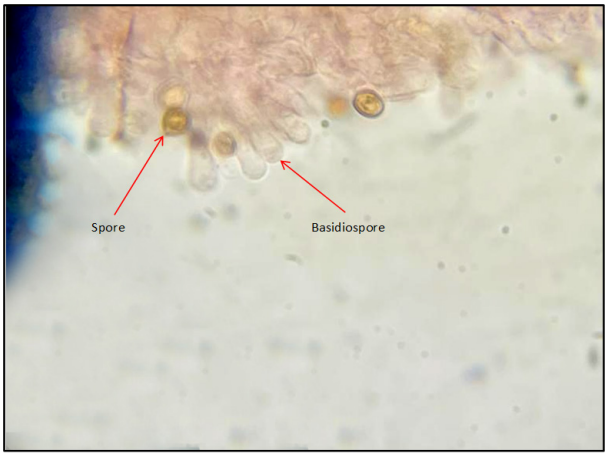


Figure 3. Microscopy determination of *P. ostreatus*, hyphae and basidiospores at 100X without dyeing

3.3 Extraction of active components (β-D-Glucan)

The results of the chromatography analysis using the HPLC technique revealed the availability for a number of active compounds based on standards (β-D-Glucan (pluran), as shown in Figures: 4, 5, 6, 7 and 8).

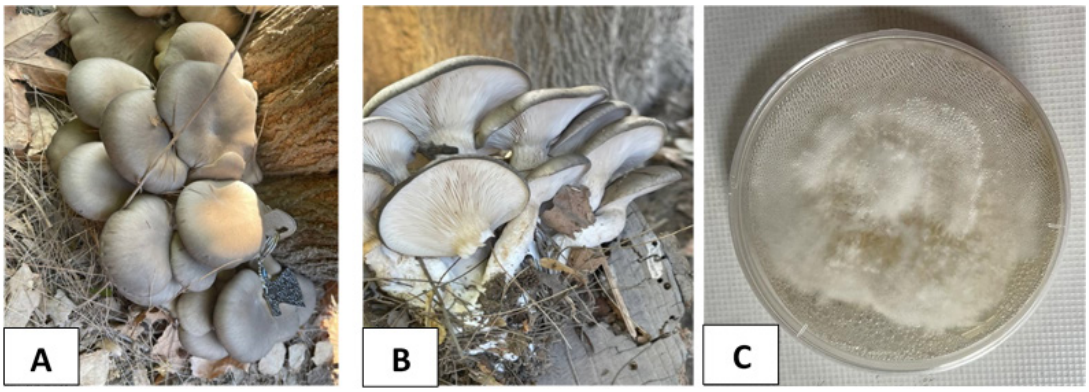


Figure 2. Phenotypic diagnosis of *P. ostreatus*
(A): The fruiting body of the selected fungal isolation; (B): The shape of the gills in fungal isolation; (C): colony of *P. ostreatus* on PDA

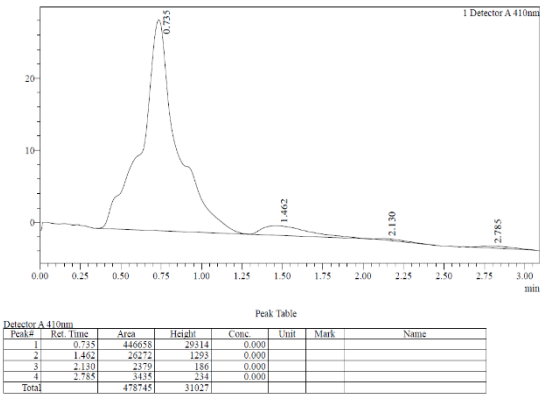


Figure 4. The concentration of β-D-Glucan (pluran) in aqueous extract in Mosul forests

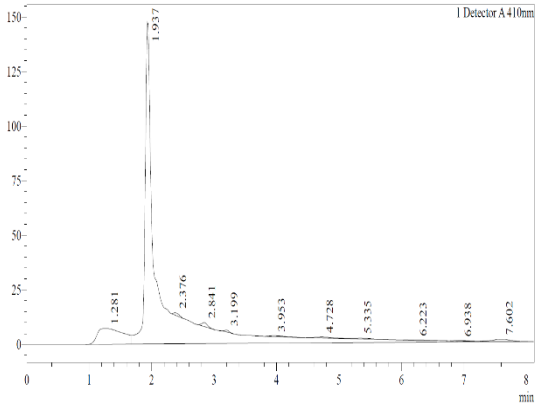


Figure 5. The concentration of β-D-Glucan (pluran) in aqueous extract in Alhadba' farms

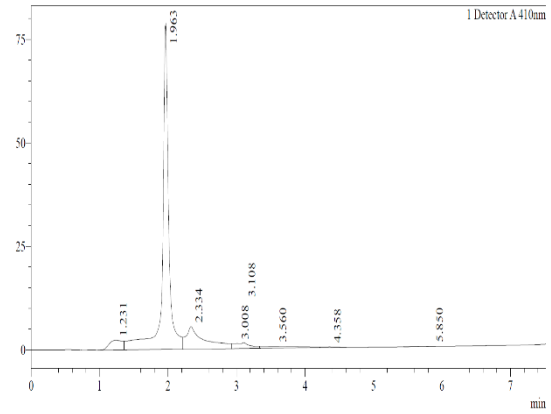


Figure 6. The concentration of β-D-glucan (pluran) in aqueous extract in Rashidia

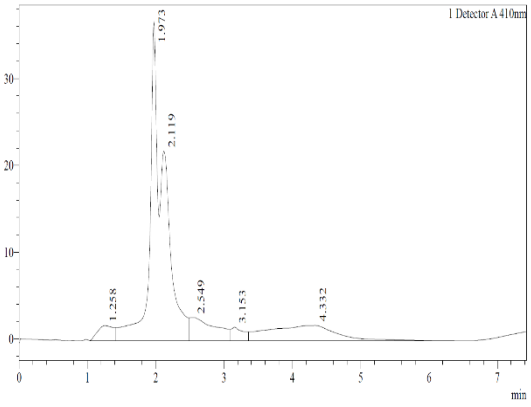


Figure 7. The concentration of β-D-Glucan (pluran) in aqueous extract in Alsalam farms

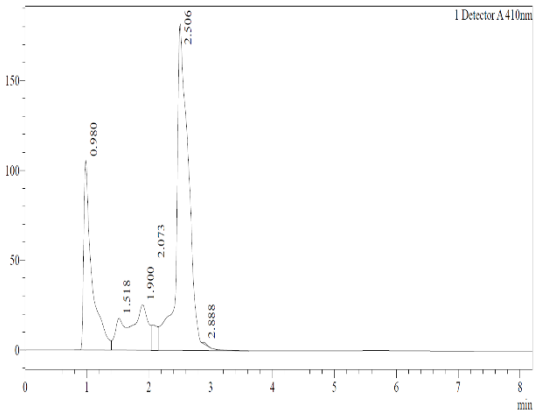


Figure 8. The concentration of β-D-Glucan (pluran) in aqueous extract in Alsalamia

3.3.1 Evaluation the concentration of active components from *P. ostreatus* in aqueous extraction

Active components from *P. ostreatus* are shown in Table 2. The results reveled that β-D-Glucan (pluran) was higher concentration in Mosul forest (9.842 µg/mL), followed by Alhadba' farms, Rashidia region, and Alsalam farms, (3.171, 3.045, and 2.372 µg/mL) respectively. Lower levels of β-D-Glucan (pluran) (0.388 µg/mL) was found in Alsalamia region.

The differences in concentrations of compounds and their quality can be attributed to many variable as: geographical and climatic conditions, which are represented in temperatures and the amount of rainfall, as well as the age of fruiting bodies, which

have a major role in the yield of secondary metabolic results, as well as the nature of developing organic matter [27]. Based on our findings we can confirm that *P. ostreatus* is a useful fungus that exists in Iraq, and it is an important part of the ecosystem.

3.3.2 Evaluation the concentration of active components from *P. ostreatus* in petroleum ether extraction

Table 3 shows the results of petroleum ether extracted of active components from *P. ostreatus*. The results revealed that β-D-Glucan (pluran) was a higher concentration in the Mosul forest (265.205 µg/mL), followed by the Alsalamia region, Rashidia region, and Alhadba' farms (143.368, 142.487, and 99.775 µg/mL) respectively. Lower levels of β-D-Glucan (pluran) (31.204 µg/mL) was found in Alsalam farms.

The medicinal active compounds of *Pleurotus ostreatus* has several active metabolites that can be employed as antioxidants, anti-inflammatory agents, cholesterol-lowering agents, and anti-cancer agents. The aqueous extract of *P. Ostreatus* is one of the therapeutic extracts under development [27]. The drying procedure, the choice of the solvents employed, and the ratio of the solvents have a significant impact on the quality of herbal preparations, such as the ethanol extraction. Ethanol is widely used as a universal solvent in the extraction due to its ability to dissolve both polar and nonpolar compounds.

In conclusion, we note from the Tables 2 and 3 that the highest curve height for β-D Glucan (pleuran) was found in the isolates from the Rashidia site (at a retention time of 1.012 in the extract of petroleum ether, and the lowest height was at isolation from the integrity site, the retention time reached 0.980 P2). Compared to the standard sample, the results indicate that it is almost no in the aqueous extract in all areas under study.

Many factors can affect the molecular weight (MW) of β-Glucan, such as food processing and the source of β-Glucan. Heating, in particular, can decrease the MW of β-Glucan and therefore decrease its viscosity inside the glycemic index (GI) [28]. Different sources of β-D-Glucan may also have different MW and viscosity. It has been noted that the MW among oat varieties differ, which leads to the assumption that the MW can also vary among cereal grain types [29]. β-D-Glucan from oat and barley also differ in their solubility which can directly affect intestinal viscosity [30] with a higher proportion of the total fraction being soluble in oats and barley than in wheat which is likely due to the chain length (shorter chains being more soluble) [31]. As mentioned previously, the basic structure (linkage pattern) may account for the differences that occur in the properties of β-glucan, specifically solubility and viscosity.

3.4 Biological efficacy of β-D Glucan (pleuran) against pathogenic fungi

β-D Glucan (pleuran) had a relatively the higher effect on *Candida* spp. under study compared to other active compounds, especially *Candida lusitaniae*. It highest inhibitory concentration was at 200 µg/mL at OD

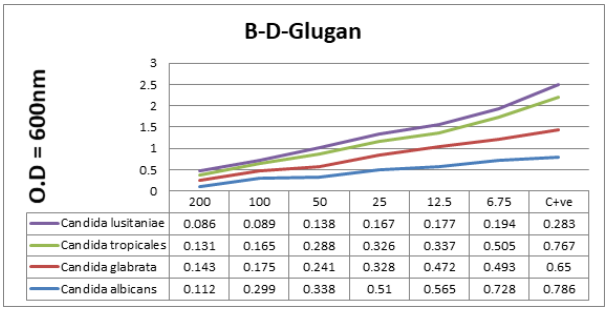
Table 2. Concentrations of active compounds in aqueous extraction by HPLC technique

Site	Ret. time (min)	Area	Concentration (µg/mL)
Standard (β-D-glucan)	**0.735**	**446658**	**40**
(β-D-glucan) found:			
Alsalamia	0.947	2089	0.388
Rashidia	1.236	24521	3.045
Alhadba' farms	1.231	32118	3.171
Alsalam farms	1.258	28028	2.372
Mosul forest	1.281	211650	9.842

Table 3. Concentrations of active compounds in petroleum ether extraction by HPLC technique

Site	Ret. time (min)	Area	Concentration (µg/mL)
Standard (β-D-Glucan)	**0.735**	**446658**	**40**
(β-D-glucan) found:			
Alsalamia	0.980	960155	143.368
Rashidia	1.000	1261220	142.487
Alhadba' farms	1.012	865373	99.775
Alsalam farms	0.974	1550218	31.204
Mosul forest	0.999	200514	265.205

0.086, while OD value was the lowest inhibition for *Candida glabrata* 0.143 at the same concentration. The MIC effect for all fungi was 12.5, as shown the Figure 8.



	200	100	50	25	12.5	6.75	C+ve
Candida lusitaniae	0.086	0.089	0.138	0.167	0.177	0.194	0.283
Candida tropicales	0.131	0.165	0.288	0.326	0.337	0.505	0.767
Candida glabrata	0.143	0.175	0.241	0.328	0.472	0.493	0.65
Candida albicans	0.112	0.299	0.338	0.51	0.565	0.728	0.786

Figure 8. Biological Efficacy of β-D-Glucan (pleuran) against pathogenic fungi

In the study conducted by *Gutef et al.*, [32], on the evaluation of the antibacterial potential of fruit body extracts of pleurotus ostreatus in vitro and in vivo study, it was found significant difference ($P < 0.05$) between the treated group and negative control, but not with the positive control. This may due to the bioactive compounds existing in the fruiting bodies of mushroom methanol crude extract which has the most potential therapeutic effect.

4. Conclusions

- We conclude through the results, *Pleurotus ostreatus* contain many effective compounds of medical and pharmaceutical importance, the most important of which is β-D-Glucan (pluran).
- The results in this study showed that β-D-Glucan (pluran), extracted and purified from *Pleurotus ostreatus* mushroom efficacy against following microorganisms: *pathogenic E. coli*, *Staphylococcus aureus, Bacillus subtilis,* and *Proteus vulgaris*, as well as against: *Candida albicans*, *Candida nakaseomyses*, *Candida tropicales*, *and Candida lusitaniae*.

5. References

[1] Kumar A. (2021). *Role of iron-phenolic acid interaction in colour development during sugar processing-a review study*. In: Kumar A., Singh K. R. Rao Y. (Eds.), Recent trends in chemical sciences and environmental science, Thanuj International, Rasipuram, India, pp. 135-149.

[2] Wang N., Wang Z. Y., Mo S. L., Loo T. Y., Wang D. M., Luo H. B., Yang P. D., Chen L. Y., Shen G. J., Chen J. P. (2012). *Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer*. Breast cancer research and treatment, 134, pp. 943-955.

[3] Jakubczyk D., Dussart F. (2020). *Selected fungal natural products with antimicrobial properties*. Molecules, 25, (4), 911. DOI:10.3390/molecules25040911. Accessed 25 June 2021.

[4] Sulaiman I. M., Hsieh Y. H. (2017). *Foodborne pathogens in milk and dairy products: Genetic characterization and rapid diagnostic approach for food safety of public health importance*. In: Watson R. R., Collier J. C., Preedy R. V. (Eds.), Dairy in Human Health and Disease Across the Lifespan, Academic Press, Cambridge, USA, pp. 127-143.

[5] Patel S., Goyal A. (2012). *Recent developments in mushrooms as anti-cancer therapeutics: A review*. Biotech, 2, pp. 1-15.

[6] Lin P. H., Aronson W., Freedland S. J. (2015). *Nutrition, dietary interventions and prostate cancer: The latest evidence*. BMC medicine, 13, (1), pp. 1-15.

[7] Fernandes Â., Barros L., Martins A., Herbert P., Ferreira I. C. (2015). *Nutritional characterisation of Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm. produced using paper scraps as substrate*. Food Chemistry, 169, pp. 396-400.

[8] Devi K. S. P., Roy B., Patra P., Sahoo B., Islam S. S., Maiti T. K. (2013). *Characterization and lectin microarray of an immunomodulatory heteroglucan from Pleurotus ostreatus mycelia*. Carbohydrate polymers, 94, (2), pp. 857-865.

[9] Sułkowska-Ziaja K., Szewczyk A., Galanty A., Gdula-Argasińska J., Muszyńska B. (2018). *Chemical composition and biological activity of extracts from fruiting bodies and mycelial cultures of Fomitopsis betulina*. Molecular biology reports, 45, pp. 2535-2544.

[10] Mirończuk-Chodakowska I., Kujawowicz K., Witkowska A. M. (2021). *Beta-glucans from fungi: Biological and health-promoting potential in the COVID-19 pandemic era*. Nutrients, 13, (11). DOI:10.3390/nu13113960. Accessed 20 June 2022.

[11] Kumakura K., Hori C., Matsuoka H., Igarashi K., Samejima M. (2019*). Protein components of water extracts from fruiting bodies of the reishi mushroom Ganoderma lucidum contribute to the production of functional molecules*. Journal of the Science of Food and Agriculture, 99, (2), pp. 529-535.

[12] Singdevsachan S. K., Auroshree P., Mishra J., Baliyarsingh B., Tayung K., Thatoi H. (2016). *Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review*. Bioactive carbohydrates and dietary fibre, 7, (1), pp. 1-14.

[13] Sari M., Prange A., Lelley J. I., Hambitzer R. (2017). *Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms*. Food Chemistry, 216, pp. 45-51.

[14] Murphy E. J., Rezoagli E., Major I., Rowan N. J., Laffey J. G. (2020). *β-glucan metabolic and immunomodulatory properties and potential for clinical application*. Journal of Fungi, 6(4), 356. DOI:10.3390/jof6040356. Accessed 20 June 2022.

[15] Balasubramanian S., Kala S. M. J., Pushparaj T. L. (2020). *Biogenic synthesis of gold nanoparticles using Jasminum auriculatum leaf extract and their catalytic, antimicrobial and anticancer activities*. Journal of Drug Delivery Science and Technology, 57. <URL:https://doi.org/10.1016/j.jddst.2020.101620. Accessed 20 June 2022.

[16] Al-Dabbas M. M., Al-Jaber H. I., Al-Rawashdeh I. M., Al-Rawashdeh N. F., Abu Zarga M H. (2019). *Extraction, phytochemical analysis and antioxidant activity of Citrullus colocynthis fruit extract*. Int. J. Food Prop., 22, (1), pp. 141-154.

[17] Xing S., Yu W., Zhang X., Luo Y., Lei Z., Huang D., Lin J., Huang Y., Huang S., Nong F., Zhou C., Wei G. (2018). *Isoviolanthin extracted from Dendrobium officinale reverses TGF-β1-Mediated Epithelial–Mesenchymal transition in hepatocellular carcinoma cells via deactivating the TGF-β/Smad and PI3K/Akt/mTOR Signaling pathways*. International Journal of Molecular Sciences, 19, (6). DOI:10.3390/ijms19061556. Accessed 20 June 2022.

[18] Qian B. J., Liu J. H., Zhao S. J., Cai J. X., Jing P. (2017). *The effects of gallic/ferulic/caffeic acids on colour intensification and anthocyanin stability*. Food Chemistry, 228, pp. 526-532.

[19] Ren G., Xue P., Sun X., Zhao G. (2018). *Determination of the volatile and polyphenol constituents and the antimicrobial, antioxidant, and tyrosinase inhibitory activities of the bioactive compounds from the by-product of Rosa rugosa Thunb. var. plena Regal tea*. BMC complementary and alternative medicine, 18, (1), pp. 1-9.

[20] Shen P., Gao Z., Xu M., Ohm J. B., Rao J., Chen B. (2020). *The impact of hempseed dehulling on chemical composition, structure properties and aromatic profile of hemp protein isolate*. Food Hydrocolloids, 106. <URL:https://doi.org/10.1016/j.foodhyd.2020.105889. Accessed 20 June 2022.

[21] Liu Y., Su H., Song H. L. (2018). *Comparison of four extraction methods, SPME, DHS, SAFE, versus SDE, for the analysis of flavor compounds in Natto*. Food Analytical Methods, 11, pp. 343-354.

[22] Delma F. Z., Al-Hatmi A. M., Buil J. B., van der Lee H., Tehupeiory-Kooreman M., de Hoog G. S., Meletiadis J., Verweij P. E. (2020). *Comparison of MIC test strip and Sensititre YeastOne with the CLSI and EUCAST broth microdilution reference methods for in vitro antifungal susceptibility testing of Cryptococcus neoformans*. Antimicrobial Agents and Chemotherapy, 64, (4). DOI:10.1128/AAC.02261-19. Accessed 21 June 2022.

[23] Sultan S. M., Saady A. M., Irzoqy M. E. (2018). *A Comparative Study of the Effect of Alcoholic Extract of Turmeric Plant in Inhibiting the Growth of Candida Albicans*. International Journal of Engineering and Technology, 7, (4.37), pp. 12-16.

[24] Ramos Y., González A., Sosa-Acosta P., Perez-Riverol Y., García Y., Castellanos-Serra L., Gil J., Sánchez A., González J. L., Besada V. (2019). *Sodium dodecyl sulfate free gel electrophoresis/electroelution sorting for peptide fractionation*. Journal of separation science, 42, (24), pp. 3712-3717.

[25] Murugesan A. K., Kaviyarasan V., Muthumary J. (2017). *Morphological and Molecular characterization of Pleurotus flabellatus from Paechiparai forest of Tamil Nadu, India*. Kavakka, 48, pp. 70-75.

[26] Kimic K., Mirzwa-Mroz E., Szyndel M. S. (2023). *Diagnosis and recommendations for management of trees and shrubs in green squares in Warsaw based on research on fungal diseases*. Trees, 37, (1), pp. 161-175.

[27] Xu D., Wang Y., Keerio A. A., Ma A. (2021). *Identification of hydrophobin genes and their physiological functions related to growth and development in Pleurotus ostreatus*. Microbiological Research, 247. <URL:https://doi.org/10.1016/j.micres.2021.126723. Accessed 22 June 2022.

[28] Gogoi P., Chutia P., Singh P., Mahanta C. L. (2019). *Effect of optimized ultrasound-assisted aqueous and ethanolic extraction of Pleurotus citrinopileatus mushroom on total phenol, flavonoids and antioxidant properties*. Journal of Food Process Engineering, 42, (6). <URL:https://doi.org/10.1111/jfpe.13172. Accessed 22 June 2022.

[29] Sun Y., Zhang M., Fang Z. (2020). *Efficient physical extraction of active constituents from edible fungi and their potential bioactivities: A review*. Trends in Food Science and Technology, 105, pp. 468-482.

[30] Angelini P., Girometta C., Tirillini B., Moretti S., Covino S., Cipriani M., D'Ellenaa E., Angelesa G., Federicia E., Savino E., Cruciani G., Venanzoni R. (2019). *A comparative study of the antimicrobial and antioxidant activities of Inonotus hispidus fruit and their mycelia extracts*. International Journal of Food Properties, 22, (1), pp. 768-783.

[31] Milovanovic I., Zengin G., Maksimovic S., Tadic V. (2021). *Supercritical and ultrasound-assisted extracts from Pleurotus pulmonarius mushroom: Chemical profiles, antioxidative, and enzyme-inhibitory properties*. Journal of the Science of Food and Agriculture, 101, (6), pp. 2284-2293.

[32] Gutef A. H., Al-Attraqchi A. A., Tawfeeq A. T., Saheb H. B. (2020). *Evaluation of antibacterial potential of fruiting body extracts of Pleurotus ostreatus in vitro and in vivo study*. Drug Invention Today, 14, (6), pp. 854-863.