Biological Activity of Phenolic Compounds Determined by HPLC Method From Pleurotus sp. Against Some Types of Pathogenic Bacteria and Fungi, As Antioxidants

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Abstract

The results show that the phenolic compounds are diagnosed through fungus. Pleurotus sp. has an effect in inhibiting the growth of some pathogenic bacteria and fungi, using different concentrations and well diffusion methods. The results show the best concentration is at 100 mg/ml, inhibition diameter 27 ml against Aspergillus niger, and the lowest inhibition area is 11 mg/ml against Candida albicans, and the phenolic compounds gave effective Antioxidant, the highest effect reached at a concentration of 400 mg/ml is 72.08, compared to the standard antioxidant, Vitamin C.

Keywords: Phenolic Compounds, Antioxidant, Antifungal, Antibacterial, Mushroom

Introduction

Pleurotus sp., known as the oyster mushroom, is marked by its high nutritional value. It belongs to the Basidiomycota class fungi (Patel *et al*., 2012). It is differentiated by phenotypic characteristics that differ from the rest of the food fungi and is characterized by its relatively simple cultivation method due to its enzyme compounds that decompose a variety of cellulosic waste (Mane *et al*., 2007; Li, N *et al*., 2015). This mushroom has a high nutritional value because its fruit bodies contain a high percentage of proteins, vitamins, saturated fatty acids, mineral elements, and phenolic compounds. It is also low in fat and free of cholesterol (Al-Faqeeh *et al*., 2020). It is considered an alternative source for obtaining natural products and showed high antimicrobial efficacy (Kim *et al*., 2022). Oyster mushroom needs to manufacture natural compounds (secondary metabolites) with an effective anti-microbial activity that grows in their natural environment to survive. This encourages the isolation of these natural compounds and their use as antifungals and bacteria, such as terpenes, alkaloids, and phenols (Lee *et al*., 2020). The control of microbes, especially pathogens, has become a real problem for humans, especially in recent years, despite the presence of manufactured chemicals that are effective in preventing the growth of Sensitive microorganisms. However, the resistance of pathogens to these chemical compounds has become clear. What complicates matters further is the presence of the side effects associated with the use of these antibiotics on the vital organs of humans such as the pancreas and spleen, as well as their clear impact on the immune system, which represents the natural balance in disease resistance (Xai, 1998). This problem prompted researchers to eagerly find alternative natural sources. To fight drug-resistant pathogens used and to develop new antimicrobial materials from various sources such as arains (Roman *et al*., 2020). The high content of antioxidants found in basidia fungi can be used as nutritional supplements that provide the human body with energy as well as protect cells from the damage of oxidative stress (Sami *et al*., 2021). Its ability to fortify the body against common diseases of age. Antioxidants have multiple functions that meet most of the human body's needs of prevention, healing, and restoration of body tissues and cells. They can be obtained from Basidian fungi known as potential sources of phenols that can enhance the body's antioxidant defense systems to prevent or treat diseases related to oxidative stress (Khongdetch *et al*., 2022).

Material and methods

Sample collection and work site

The oyster mushroom *Pleurotus osreatus* is produced through cultivation in certain agricultural conditions to produce fruiting bodies of the mushroom. The mushroom spawn is obtained from the Duhok region to conduct experiments on it. The experiment is conducted at the University of Mosul, College of Education for Pure Sciences, in the Natural Products Laboratory in the Department of Biology

Cultivation of the oyster mushroom Pleurotus osreatus

Preparation of the culture medium:

Three culture media are prepared to grow the oyster mushroom *Pleurotus* sp. Of the following components from the medium of wheat straw, sawdust, and corn cobs, urea and gypsum are added to each of them. The components are mixed by four replications for each medium (Vedder, 1978). The components are moistened with tap water and left for 18 hours until saturation with water, and left to

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get rid of the excess water. On the twelfth day, urea, and gypsum (calcium sulfate) are added to them, then the components are collected in piles with continuous stirring every three days. The fermentation period lasts from the sixteenth day. Until the eighteenth day with hydration when needed and the process continued for 21 days, after which the media is pasteurized (Beyer 2003).

Mushroom classification

The fungus is classified as *Pleurotus osreatus* according to the phenotypic characteristics of the fruiting body and according to the available descriptive keys (1988, Large & Baroni; 1981, Phillips; 1961, Hard). Stem diameter and length, cab length, spore print, shape, and colour, fruiting body and the presence of the ring and fascia.

Extraction by Soxhlet continuous extractor (Soxhlet)

The extraction process is carried out using a Soxhlet device and using the successive solvent system (hexane, ethyl acetate, ethanol) as shown in picture No. (12), where 100 g of fungal powder is added per 1000 ml of solvent, i.e. 100 g / 1000 ml, and the extraction process continues for some time between (48-72) hours for each solvent until the color of the solvent disappears in the device to get the extract, which is a mixture consisting of the fungal extract with the solvent. After this stage, the solvent is separated from the extract using the rotary vacuum evaporators to get (25) ml, and the crude extract is placed in dark-colored glass bottles and kept in the refrigerator until use (Harborne, 1998).

High-performance liquid chromatography (HPLC)

HPLC measurements are carried out at Samarra Pharmaceutical Company / Intravenous Solutions Factory in Nineveh using an HPLC device from Agilent Technologies, an American company, using a Supelco C18 separation column (25mm x 4.6mm x 5mm) at room temperature of 25C. As the solvent used in the Mobile phase, it is a mixture of polar solvents with water such as methanol, MeOH, and acetonitrile (20:80). After it is filtered and gases are expelled from it using an ultrasonic device (sonicator) at a flow rate of 1 = flow rate ml/min and a wavelength of 280 = wavelength nm.

Microorganisms used in the study

Escherichia coli, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans* are obtained from the laboratory of the research unit of the Department of Life Sciences/College of Science and College of Education/the University of Mosul.

Preparing the concentrations of the active ingredients and sterilizing them for the studied fungi

Active compounds concentrations are prepared from the fungus extract to obtain 200 g/ml as a standard concentration to prepare the necessary dilutions, by dissolving 1 g of the raw active compounds and phenolic compounds with 5 ml of (DMSO) Dimethyl sulfoxide, then sterilizing the extract used, which is used by the drilling method, and it is sterilized by the sterilization filter. 0.22 µl Millipore microfilters (1998, Shareef).

Well method

The inhibitory activity of the active components in the growth of studied fungi and bacteria are tested by sensitivity test by agar well diffusion method according to Peres and Bazerque's (1991) method. 5 microliters of each concentration of the extract of *Agaricus* sp. and *Pleurotus* sp, as well as the control are immersed in sterile distilled water. The dishes for all fungi are incubated for 7 days at a temperature of 26 C, while the dishes containing bacteria are incubated at a temperature of 37 C for 24 hours. The potency of each concentration of extracts is determined by measuring the diameter of the inhibition zone around each hole (Sridhar, 2011).

Detection of antioxidant activity

This test is conducted at the Biotechnology Research Center, Al-Nahrain University.
The DPPH test (2,2 Diphenyl-2-PicryHydrazyl) is used to determine the extent of the antioxidant activity of the isolated and identified compounds from the fungi approved in the study, according to the method used by (Rajesh & Natvar 2019).

Test principle

DPPH is a solution with a dark violet color that can stabilize free radicals, it turns yellow when exposed to antioxidants. The effectiveness of the fungal extract is quantitatively estimated using spectrophotometry at a wavelength of 518 nm, and the percentage of sample effectiveness is calculated through the following law:
Sample efficacy percentage = 100*(Ab sample-Ab-ev)/(Ab-ev)
Since:-
Ab-ev: represents the absorbance ratio of the negative control sample.
Ab sample: represents the absorbance ratio of the sample.
1. DPPH solution 2. Methanol solution 3. Ascorbic solution.

Methods

The antioxidant activity of the identified active compounds is measured and included:
1- A series of dilutions of (g | mi400, 200, 100, 50, 25) are prepared and 10 ml of the diagnosed active compounds are added to each of them, Methanol: DMSO in a ratio of 9:1.
2- Dissolve 0.02 g of DPPH in 100 ml of Methanol: DMSO in a ratio of 1: 9
3- 0.3ml of DPPH is added to each tube.
4- A negative control tube is prepared, Blank, containing DPPH solution and Methanol solution: DMSO only.

5- Sample tubes containing DPPII solution and Methanol DMSO solution are prepared, then the concentrations that are prepared from the fungal extracts are added at a rate of (3) bis for each concentration.

6- A wave-control tube containing DPPH solution, Methanol solution: DMSO, and Ascorbic acid solution (vitamin C) is prepared.

7- The results are read on a spectrophotometer at a wavelength of 518 nm and the annual percentage is calculated according to the above-mentioned equation.

Statistical analysis

The data are statistically analyzed using the Minitab computer system according to the analysis of variance ANOVA, and the arithmetic means are compared with the DNCN polynomial test with a significant difference at a probability level of $P < 0.05$ and a significant difference at the level of $P < 0.01$.

Results and discussion

Identification of phenolic compounds separated from Pleurotus sp fungus using high-performance liquid chromatography (HPLC).

The phenolic compounds (gallic acid, salicylic acid, vanillic acid, rontin) are identified in *Pleurotus* sp. as shown in Table (1)

Table (1) Values of RT for a number of phenolic compounds separated from *Pleurotus* sp. fungus and RT for standard compounds using HPLC technology.

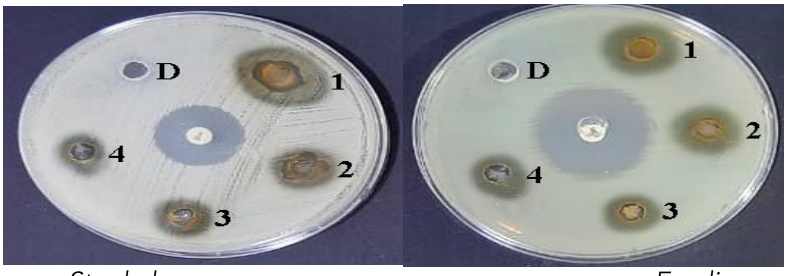
Sample Detention time (min) for the standard sample	Detention time (min) for the fungal	Diagnosed compound
2.019	2.029	Gallic acid
2.405	2.388	Salicylic acid
3.031	3.041	Vanillic acid
3.911	4.011	Runtin

The phenolic compounds diagnosed from *Pleurotus* sp. showed effectiveness against Staph aureus at concentrations of 100, 75, 50, 25 mg/ml, 12, 15, 17, and 12, respectively, while the inhibition rate against E.coli is 16, 15, 12, 12 for the same concentrations, while the percentage of inhibition against *Candida albicans* is 11, 15, 14, 13 mm for concentrations 100, 75, 50, 25 mg/ml, and it gave the highest percentage of inhibition towards *Aspergillus niger*, where the percentage of inhibition was0, 0, 16, 27, mm respectively, as shown in Table (2) and Fig. (1).

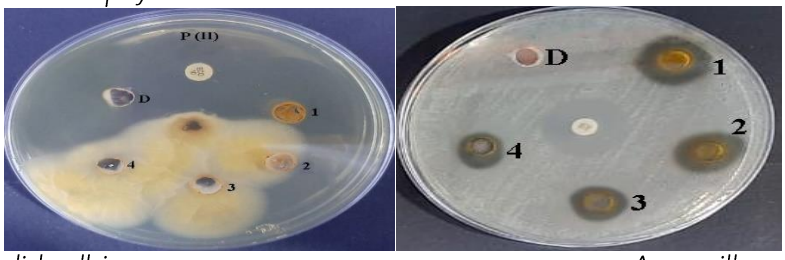
Table (2) Biological activity of phenolic compounds diagnosed from *Pleurotus* sp. against some types of bacteria and fungi.

Aspergillus niger	*Candida albicans*	*E.coli*	*Staph aureus*	Bacteria and fungi concentration mg/ml
27	15	16	17	100
14	14	15	15	75
0	13	12	12	50
0	11	12	12	25

- Average diameter of the inhibition circle according to the use of three replicates for each bacteria and fungus.
- The diameter of the damping circle is measured in mm.
- 0 has no effect.



Staphylococcus aureus *E.coli*



Candida albicans *Aspergillus niger*

Figure (1) shows the biological activity of phenolic compounds diagnosed from P. ostreatus against some types of bacteria and fungi with different concentrations:

1- 100 mg/ml
2- 75 mg/ml
3- 50 mg/ml
4- 25 mg/ml

D (control) DMSO solvent

Phenolic compounds have an inhibitory effect on top of bacterial and fungal isolates (Rosa *et al*., 2020). The reason for the inhibition of phenolic compounds on Staphylococcus aureus and E.coli is due to their containing hydroxyl groups, which inhibit the mechanism of action of enzymes through their oxidized groups that interact with Sulfa or hydroxyl group that interacts with Sulfa or hydroxyl group. Proteins are teratogenic and degradable.

The test results also showed that the phenolic compounds (gallic acid, salicylic acid, vanillic acid, rontin) separated and identified from the fungus. *Pleurotus* spp. showed an antioxidant activity that ranged between (72.08%-26.77%) at concentrations (400—25) µg/ml, respectively. In addition, the results showed that the IC50 value of these compounds is (122.4) as shown in Table (3) and Fig. (2). Phenolic compounds are of great medical importance, due to their antioxidant and anti-inflammatory activity, among others, such as the catechol compound and it has antioxidant effects as it contains the NO2 group, which plays an important role in the elimination of free radicals (Lu *et al*., 2016), as well as vanillic acid is an important phenolic compound that exists in a range of A wide range of medicinal plants is used as a flavoring agent, in addition to its antioxidant and anti-inflammatory activity, as well as anti-mutagenic effects (Punvittayagul *et al*., 2021). Calic acid is known for its antioxidant effects (Lo *et al*., 2010) and rutin is a natural antioxidant and anti-inflammatory flavonoid, as it interacts with free radicals and neutralizes their negative effects (Zheng, 2022).

Table No. (3) Antioxidant activity of phenolic compounds diagnosed from mushrooms. *Pleurotus* sp. using the DPPH. test

The sample	Concentration mg/ml					IC50
	25	50	100	200	400	
Vitamin C	±52.20 1.408	±57.91 3.423	±65.20 2.567	±78.67 1.850	±85.03 0.5989	75.67
Phenolic compounds diagnosed for fungus Pleurotus sp	±26.77 0.7146	±29.01 1.892	±42.21 1.771	±59.54 1.597	±72.08 1.242	122.4

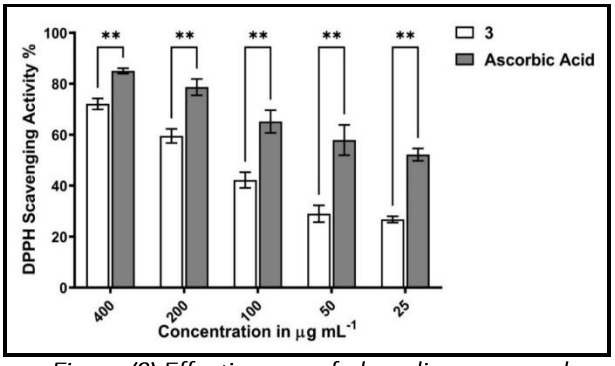


Figure (2) Effectiveness of phenolic compounds diagnosed fungus. Pleurotus sp. as an antioxidant compared to ascorbic acid as a standard sample according to the DPPH method.

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