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Effect of Edibl mushroom extracts *Agaricus sp.* , *Pleurotus sp.* On fungi isolated from soil and diagnosed using HPLC technology

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Abstract:

Ethanolic and aqueous extracts of the edibl fungi *Agaricus sp.* and *Pleurotus sp.* The study showed an effective effect against the growth of fungi isolated from the soil. The results showed a decrease in the biomass weight of the study fungi with varying weights. The fungi were most affected by the ethanolic and aqueous extracts, namely *P. notatum* and *T.harzianum*, where *P. notatum* had the lowest biomass weight at the extract. Ethanol of the fungus *Agaricus sp.* The aqueous extract of the fungus *Pleurotus sp.* With a weight of 0.01 g, it gave the diagnosis of ethanolic and aqueous extracts of the edibl mushroom *Agaricus sp.* and *Pleurotus sp.* Used in the study using HPLC technology on the following phenolic compounds: (Gallic acid, Chlorogenic, Apigenin, Keampferol, Quercetin, Catechin). These compounds are considered antifungal, antimicrobial and antioxidant compounds.

Keywords: edibl mushrooms, antifungal, HPLC technology

Introduction :

Fungi are eukaryotes and belong to a kingdom of their own called Myceteae: Kingdom (Abu Hila, 1987). Medicinal mushrooms have a history of use in traditional oriental remedies in Japan, China, Korea, and other Asian countries, based on preparations derived from edible mushrooms. Edible mushrooms have been used for many years in Eastern culture as edibl due to their distinct aroma and texture (Manzi et al., 1999). In a study conducted by (Jonathan, 2003) that the types of edibl mushroom *Agaricus sp.* and *Pleurotus sp*. It has been shown to possess anti-fungal, anti-bacterial, anti-viral and anti-cancer compounds. The fruit body of

edibl mushrooms contains compounds with antimicrobial activity, and its compounds can be isolated from the fungus *Aleurodiscus sp.* , *Pleurotus sp*., *Tricholoma sp*. And others, they contain rich sources of β-glucan, Lactin, phenolic compouneds, flavonoids, terpenes, coumarins, porisin and other active compounds (Cohen and Hadar, 2002) and the nutritional qualities of edible mushrooms and the contents of the bioactive compounds they contain make mushrooms a healthy food, as mentioned by Pereira et al. ., 2012) and is rich in water, minerals, proteins, fiber and carbohydrates, as well as low-calorie foods due to its low fat content (Heleno et al., 2009; Kavishree et al., 2008).

Edibl mushrooms that have anti-pathogenic activity contain metabolites that can be used in pharmaceutical products (Pinna, 2010). In a study conducted (Alves et al., 2013) it was found that mushrooms contain quercetin, ferulic, gallic acid, And comaric acid p- These phenolic compounds have proven to have strong anti-fungal activity against many bacteria and fungi.

Material and methods:

Isolate fungi

Soil samples were collected from sandy soil in the autumn with a depth of 15 cm in Mosul city. Fungi were isolated by dilution method by mixing 1 g of soil sample in 10 ml of sterile distilled water to make microbial suspensions of (10^{-1}-10^{-10}), and dilution was used. (10^{-3} -10^{-6}) To isolate the fungi, and before the medium solidified, 1 ml of each microbial suspension (from each concentration) was added to dishes containing PDA, and the antibiotic streptomycin was added to the medium. Food at a

concentration of 1 mg / liter and with 3 replications for each dish. The dishes were incubated at a temperature of ± 25 C°. The work was carried out in the research laboratory, Department of Life Sciences, College of Education for Girls, Mosul University (Kumar, 2014).

Diagnosis of fungi isolated from soil:

After completing the growth of the isolated fungal colonies from the soil, they were phenotypically described in terms of shape, color, texture and secretion of pigments, and they were examined microscopically by taking part of the fungal growth by using a sterile needle from the edge of the fungal colony in the case of mold growth, and using a sterile Loop in the case of the growth of yeasts and their like. It was placed on a glass slide containing a drop of potassium hydroxide (KOH), and the sample was spread in the drop, then the slide cover was placed and examined microscopically to identify the characteristics of mycelium and spores and diagnosed according to the taxonomic keys.(Al-Boni, 1990; Koneman et al., 2006; Ellis et al., 2007; Kidd et al., 2016)

Preparation of Potato Dextrose Broth PDB liquid medium:

The medium was prepared by taking extract of 200 g potato and 20 g dextrose, and placed in an autoclave under pressure of 1 atmosphere and temperature of 121 °C for 20 minutes.

Collection and extraction of fungal samples:

Fungal samples of the edibl mushroom *Agaricus bisporus* were collected from the local markets in the city of Mosul, while the edibl mushroom *Pleurotus osreatus* was imported from outside Iraq from Turkey for the purpose of studying and conducting experiments on it.

Extraction:

Fruiting parts were taken from the study fungi of *Agaricus sp.* , *Pleurotus sp*. Thoroughly dried, crushed and kept in airtight containers.

Preparation of extracts for the edibl mushroom *Agaricus sp.* , *Pleurotus sp.*

25 grams of dried mushroom powder were weighed and placed in a 1 liter beaker, and petroleum ether was added to it in a volume of 200 ml to remove the fat. The baker was closed and soaked for 3 days in the refrigerator and then placed on the stirrer magnetic for 72 hours at medium speed, after which the mixture was filtered. 1.Whatman No filter paper and an alcoholic petroleum ether extract was obtained. After filtering, 200 ml of ethanolic solvent at a concentration of 70% was added and left for 72 hours on a magnetic stirrer, and then the mixture was filtered with filter paper 1.Whatman No (Le-Grand, 1988)) . Then the mixture was filtered with filter paper and the petroleum ether and ethyl alcohol extracts were concentrated by a RVE Rotary vacuum evaporator at a temperature not Exceed 40 ˚C. The precipitate was taken again and the precipitate was soaked in a beaker with 200 ml of distilled water and placed on the magnetic stirrer, at a temperature of 40 - 60 ˚C. To obtain the extract in hot water, the crude extracts were then preserved as Crude in opaque glass bottles, closed tightly and placed in the refrigerator until use (Harborne, 1998).

Preparation of Potato Dextrose Broth PDB liquid media concentrations and testing of ethanolic and aqueous extracts of *Agaricus sp.* ,*Pleurotus sp.* on fungi isolated from soil.

1 g of each extract was dissolved in 5 ml DMSO at a concentration of 200 mg/ml and (0.5, 1, 1.5, 2, 2.5) ml of each concentration was added to 50 ml glass flasks containing PD medium in volumes (18, 18.5, 19, 19,5 , 17.5,) ml, respectively. The samples were incubated in glass flasks at a temperature of 26.5 ˚C for 7 days in the shaker incubator, by filtering the culture through a sterile pre-weighted piece of gauze to obtain the mycelium (biomass) and drying it in an electric oven at 60˚C for 40 minutes to obtain a constant weight. After filtration, the weight difference was measured for each fungal sample from each concentration of alcoholic and aqueous extracts whose effect after drying was measured with three replicates (Waqas etal,. 2018).

Separation and characterization of phenolic compounds from extracts of the edibl mushroom *Agaricus sp. Pleurotus sp.*, Using High Performance Liquid Chromatography HPLC:

The samples were diagnosed by high-performance liquid chromatography (HPC) and phenolic active substances were detected in the extracts of the two edibl mushrooms *Agaricus sp.* ,*Pleurotus sp.* Acid Hidrolosis was carried out by using a heating device (sublimation) 1 g of each extract of the edibl mushroom *Agaricus sp.* ,*Pleurotus sp.* By dissolving 1 g of crude ethanolic extract in 20 ml of ethanolic solvent, and 1 g of crude aqueous extract in 20 ml of distilled water. Then the sample was obtained and 10 ml of each solvent was taken, 25 ml of dilute hydrochloric acid Hcl was added to it, and it was placed on the heating device for an hour, and left for a short period to cool. Then it was placed in a separating funnel and 25 ml

was added in two phases of ethyl acetate (ethyl acetate) and after separating it was placed in opaque sealed bottles (Harborne, 1998).

statistical analysis :

The data were analyzed according to a factorial experiment 2 * 2 * 4 * 7 * 2 using the complete random design (CRD) and the significant differences between the means of transactions were determined using the least significant difference LSD at the significance level of 0.01, the ready-made statistical analysis program SPSS was used to analyze the data.

Isolation and identification of fungi

The tested fungi were isolated from the soil and dilutions were performed on soil samples. After isolation, development and microscopic diagnosis, the presence of different genera of soil-dwelling fungi was found. *Trichoderma harzianum* was the most frequent fungus, followed by *Asperigllus sp*. and *Rhizopus stolonifer* then *Penicillium notatum* down to *Rhizoctonia solani.*

Identification of some active compounds of *Agaricus sp.* and *Pleurotus sp.* HPLC High Performance Liquid Chromatography

Ethanolic and aqueous extracts of the edibl mushroom *Agaricus sp.* and *Pleurotus sp.* Used in the study using HPLC technology on the following compounds: (Gallic acid, Chlorogenic, Apigenin, Catechin) and this is consistent with what was mentioned (Sevindik et al., 2018). According to what was mentioned (Bach et al., 2019; Garrab et al., 2019), the edibl mushroom *Agaricus sp*. and *Pleurotus sp.* Rich in phenolic compounds and

flavonoids. Among the results of the current experiment, the extracts of edibl fungi gave the compound Apigenin It is a natural flavone found in medicinal plants and has an anti-bacterial mechanism, and the results indicate that it stimulates apoptosis of bacteria and microbes, and this is what he mentioned (2020 et al., Kim).

The alcoholic extracts of edibl mushroom contain catechin, gallic acid, phenolic. While the ethanolic and aqueous extracts of the edibl mushroom used in the current study gave the following phenolic compounds: (Gallic acid, Keampferol, Catechine,) and the presence of Quercetin, an anti-pathogenic fungi, and this is consistent with a study conducted by (Oliveira et al., 2016). The presence of Keampferol, which binds to the cell wall, is one of the predominant flavonoids found in vegetables and is an active antimicrobial (Huynh et al., 2018). Catechin is a phenolic compound found mainly in tea. It is an antimicrobial and works to prevent the growth of *Candida sp.* and enhanced antifungal activity (Ning et al., 2015). The results of (Sung and Dong, 2010) indicate that chlorogenic acid shows antifungal activities against some pathogenic fungi. As for the compound Gallic acid, it showed antibacterial and antimicrobial activity and could disrupt the bacterial cell membrane and raise the accumulation of antibiotics in organisms. The minute (2019Kahkeshani et al.,).

Effect of extracts of nutritional fungi on the growth of fungal biomass in liquid media:

The effect of the ethanolic extract of the edibl mushroom *Agaricus sp.* On the growth of biomass, it was more effective on *P. notatum*, where the weight of biomass decreased at the highest concentration of 2.5 ml to 0.01

g, followed by *T. harzianum* with a weight of 0.05 g compared with the control and the rest of the concentrations. As for the aqueous extract of *Agaricus sp.* The most affected fungi was *R. stolonifer T.harzianum*, where the biomass weight decreased by 0.07 / 0.08 g, respectively, at the highest concentration of 2.5 ml compared with the rest of the fungi isolated from the soil. The effect of the ethanolic extract of the edibl mushroom, *Pleurotus sp.*, showed a significant effect on the weight of biomass among the rest of the extracts of the two fungi, as all fungi isolated from the soil showed a decrease in the weight of biomass at the highest concentration compared to the control with weights of 0.05 / 0.07 / 0.08 / 0.1 g *T.harzianum* , *P. notatum*, *A. niger*, *R.stolonifer* respectively. As for the aqueous extract of the edibl fungus *Pleurotus sp.* Where the fungus *P. notatum* gave the lowest biomass 0.01 g among the rest of the fungi and showed a clear decrease in biomass compared to the control and the rest of the concentrations.

Table (1) Effect of the ethanolic extract of the edibl mushroom *Agaricus sp.* On the weight of the biomass of fungi isolated from the soil with three replicates and for an incubation period of 7 days in liquid media.

2.5	2	1.5	1	0.5	cotrol	Concentration ml / Biomass of fungi g
.3000 cd	.3600 ab	.2600 de	.2533 de	.1500 gh	.3300 bc	*R.stolonifer*

.0500 ij	.2200 ef	.2300 ef	.3300 bc	.2200 ef	.2000 f	*T.harzianum*
.0100 j	.1200 h	.2800 cd	.0700 i	.3800 a	.3200 bc	*P. notatum*
.1900 fg	.3200 bc	.3200 bc	.4000 a	.1800 fg	.1200 h	*A. niger*

Table (2): Effect of aqueous extract of the edibl mushroom *Agaricus sp.* On the weight of the biomass of fungi isolated from soil and in three replicates for a period of 7 days incubation in liquid media.

2.5	**2**	**1.5**	**1**	**0.5**	**control**	**ml Concentration / g Biomass of fungi**
.0700 gh	.0800 gh	.0600 gh	.0800 hg	.1100 g	.2400 def.	*R. stolonifer*
.0800 gh	.0600 gh	.1100 g	.0900 g	.0300 h	.2100 ef	*T.harzianum*
.2600 cde	.4100 b	.1100 g	.4100 b	.2000 f	.5600 a	*P. notatum*
.2000 f	.2400 def	.1100 g	.2900 cd	.1100 g	.3000 c	*A. niger*

Table (3): Effect of the ethanolic extract of the edibl mushroom *Pleurotus sp.* On the weight of the biomass of fungi isolated from soil and in three replicates for a period of 7 days incubation in liquid media.

2.5	**2**	**1.5**	**1**	**0.5**	**control**	**ml Concentration / g Biomass of fungi**
.1000 efg	.1000 efg	.0800 gh	.1500 def	.3400 a	.1600 de	*R.stolonifer*

.0500 gh	.1500 def	.0400 gh	.3000 ab	.0200 h	.2100 cd	*T.harzianum*
.0700 gh	.2800 ab	.0400 gh	.0700 gh	.3100 ab	.3500 a	*P. notatum*
.0800 fgh	.0500 gh	.0600 gh	.2400 bc	.2000 cd	.2800 ab	*A. niger*

Table (4): Effect of aqueous extract of the edibl mushroom *Pleurotus sp.* On the weight of the biomass of fungi isolated from the soil with three replicates and for an incubation period of 7 days in liquid media.

2.5	**2**	**1.5**	**1**	**0.5**	**control**	**ml Concentration / g Biomass of fungi**
.2900 cd	.1200 hij	.3900 b	.4000 b	.4000 b	.5000 a	*R.stolonifer*
.1000 ij	.1400 g-j	.1800 fgh	.1900 e-h	.2400 def	.2600 de	*T.harzianum*
.0100 k	.1200 hij	.1700 f-i	.1500 ghi	.1800 fgh	.1800 fgh	*P. notatum*
.2000 efg	.1800 fgh	.0700 jk	.3400 bc	.1000 ij	.3300 bc	*A. niger*

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