Inhibitory Activity of Nutritional Mushroom Extract (Agarics Bisporus) on the Growth of the Pathological Fungus Candida Sp.

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Abstract
The results of this study showed that both alcoholic (ethanol) and aqueous (hot water) extracts of the fungus (Agaricus bisporus) had an inhibitory effect on four Candida species isolated from the mouths of infected children: Candida albicans, Candida krusei, Candida tropicalis, and Candida glabrata. The aqueous extract was recorded at a concentration of 15 mg/ml, which was the highest inhibition ratio of all species. If the diameter of the inhibition zone was 19 mm against Candida krusei, 17.5 mm against Candida glabrata, 16.5 mm against Candida albicans, and 15 mm against Candida tropicalis, but in the case of alcoholic extract (ethanol), a concentration of 15 mg/ml gave the highest inhibition ratio of all isolated Candida species, where the inhibition diameter was 14.4 mm against Candida tropicalis, 13.8 mm against Candida krusei and gave 13.6 (mm) against Candida glabrata and finally 13.1 (mm) against Candida albicans. The analysis of the phenolic compounds of food mushrooms in the HPLC device showed the emergence of many compounds, including Apigenin, ferulic, gallic acid, hydro benzoic acid, qurcetine, Rutine.
Keywords: Mushroom extracts, Candida, inhibition, HPLC, phenolic compounds

1. Introduction

Candida albicans is a common symbiotic fungus that colonizes the oral cavity, pharynx, digestive tract, vagina, and skin of healthy individuals. For 50% of the population, the various clinical manifestations of Candida types range from localized superficial mucocutaneous disorders to invasive diseases involving multiple organs of the body, which in turn are life-threatening. (Talapko et al., 2021). Fungal infections are a major factor in infectious disease-related deaths worldwide. As Candida species are one of the most common causes of invasive fungal disease, (Lee et al., 2021), the exploration of natural products as a source of new human therapies is of great importance Because of their high therapeutic potential against a wide range of diseases, medicinal plants and the discovery of new drugs have a strong correlation. (Khurshid et al., 2022) that Candida is a normal flora within the body. (Akortha et al., 2009), but it becomes opportunistic and infects humans when the immune system is weakened, such as by taking chemotherapy for people with cancer, organ transplantation, or diabetes (Saporiti et al., 2015). Fungi must meet four criteria for host exploitation, including the ability to grow at or above mammalian body temperature, the ability to reach internal tissues by penetrating or avoiding host barriers, tissue analysis and absorption of their components, and their potential to evade the host's immune defenses (M. Liebana-Jordan et al. 2021).
Candida albicans appear in several morphological forms (blastospores), pseudo hyphae, and hyphae). The spores divide asexually by budding. During this process, new cellular material is formed on the surface of the spores. The new bud grows from a young sporophyte, often far from the site of the birth scar, after which the growth phase begins. After the end of the growth phase, the cells divide, where the daughter separates from the mother cell, and among the factors that affect this division are a temperature higher than 37 ° C, an alkaline pH, the presence of serum, and high concentrations of carbon dioxide [(Talapko et al., 2021)
In recent years, people have become more interested in plant extracts because they are full of natural antioxidants like vitamins, phenolic compounds, and flavonoids (Hoang et al., 2021). Edible mushrooms are an important source of food for people because they are low in calories and high in carbs, proteins, compoundsolic acids, and flavonoids (Hoang et al., 2021). Edible mushrooms are an important source of food for people because they are low in calories and high in carbs, proteins, and compounds. phenolics, dietary fiber, polyunsaturated fatty acids, minerals, and vitamins (Ramos et al., 2019). These compounds have a wide range of therapeutic effects and can act as immune-modulators, antivirals, anticancer agents, anti-inflammatory agents, and antioxidants; regular consumption of edible mushrooms protects against a wide range of diseases. Because it has all the nutrients a person needs, it is enough for one person. So, mushrooms that people can eat can be thought of as natural antibiotics for many diseases and cancers. Assemie & Abaya, 2022) Mushrooms can be found in many different places, from forests to deserts. So far, only 20 of the 2,000 species of mushrooms that can be eaten are grown

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commercially (Tanimola & Adedokun, 2020).
Mushrooms live in a variety of places, from forests to deserts, where they can be found, and so far, out of the 2,000 known edible species of mushrooms, only 20 are commercially grown (Tanimola & Adedokun, 2020).

2. Material And Methods

Sample collection

The fungus (Agaricus bisporus) was obtained from local markets, and the origin of the fungus was Iraq (Erbil). As for the source of pathogenic fungal isolates, Candida sp., they were obtained from the mouths of children under the age of five from hospitals in the city of Mosul, and they were diagnosed by microscopy and laboratory examination using diagnostic tests and differential media.

Preparation of *Agaricus bisporus* extract

Alcoholic extraction (ethanol): Grind 25 g of food mushrooms and place the powder in a 1-liter baker with 500 ml of petroleum ether solvent. Place the mixture on the magnetic stirrer device and leave for 72 hours before filtering through filter paper Whatman No. 1. take the precipitate and put it again in a baker with a capacity of 1 liter and add 500 ml of 70% ethyl alcohol concentration. It is placed on the magnetic motor for 72 hours (LeGrand et al., 1988), after which the mixture is leached, and the extracts of petroleum ether and ethyl alcohol are concentrated by the RVE rotary vacuum evaporator at a temperature of 40 °C.

Hot Aqueous Extraction

The precipitate is taken, 400 ml of distilled water is added to it, and it is placed on a magnetic stirrer at a temperature of 60 °C to obtain the hot extract (Harbourne 1984) for a period of 72 hours. The filtrate is then placed in a Lyphalaizer to obtain the extract in a dry form.

Candida Yeast Development

Sabouraud dextrose agar (SDA) medium was prepared according to the instructions of the supplying company (HI Media) by dissolving 14.5 g of the medium in 250 ml of sterile distilled water and pouring it into Petri dishes. Part of the sample was attached to a loop and placed in a previously prepared physiological saline solution, and its turbidity was compared with MacFarland's solution of 0.5 × 108 (cells / ml, thus obtaining a suitable yeast suspension for testing.

Well Diffusion Method

The inhibition activity of the alcoholic (ethanol) and aqueous extracts of the fungus A. bisporus was estimated by digging in the agar, as an inoculum was taken by 0.1 ml of the yeast suspension and grown on a medium (Sabouraud agar). Repeats for each type of Candida sp. Then holes were made with a cork bore with a diameter of 6 mm, where a central hole and five peripheral holes were made with equal dimensions, and then the extracts were placed in the holes in the periphery, 1 gm of the extract was dissolved in 5 ml of DMSO (Dimethyl sulfoxide), i.e. Equivalent to 200 mg / ml, then the following concentrations were prepared (5, 10, 15, 20, 25) mg / ml, and each concentration was placed in a hole and sterile distilled water was put in the central hole, which represents the control sample, then the dishes were incubated at a temperature of 37 M for a period of 24-48 hours, then the inhibitory diameters were measured around the pits containing the extract at different concentrations and measured by a graduated ruler and the average was taken.

Using high-performance liquid chromatography (HPLC) to detect active compounds

High-performance liquid chromatography (HPLC) analysis was performed on a SYKAMN HPLC system (Germany) equipped with a C18-ODS column (250 × 4.6 mm, 5 µm). Samples (100 µl) were injected into the system. The mobile phase consisted of 95% acetonitrile plus 0.01% trifluoroacetic acid (solvent A) and 5% acetonitrile plus 0.01% trifluoroacetic acid (solvent B) at 1 mL/min. The gradient programme was as follows: 10% A from 0–5 minutes; 25% A from 5-7 minutes; 40% A from 7–13 minutes; then back to the initial conditions. The detection of phenolic compounds was performed using a UV-visible detector at 278 nm.

Statistical Analysis

The statistical analysis was done with the SPSS program. Duncan's multiple range test was used to separate the coefficients because different letters of the alphabet made a big difference between the coefficients below a 5% probability level.

3. Results and Discussion

Inhibitory effectiveness of crude extracts of *A. bisporus* against Candida

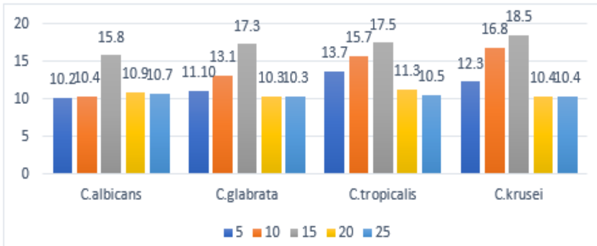


Figure (1) Effect of hot aqueous extract on Candida species and concentrations (5-10-15-20-25) mg/ml with a 48-hour incubation period

Both aqueous and alcohol (ethanol) extracts of the fungus (*A. bisporus*) showed that they were harmful to all types of Candida when they were dug up on agar (agar well diffusion). The fungus makes antimicrobial receptors that can be used to treat human diseases. The results of the current study showed that the hot aqueous extract at a concentration of 15 mg/ml was the most effective at stopping all types of *Candida*. It stopped *C. krusi* the best, with a diameter of inhibition of 19 mm, followed by *C. glabrata*, with a diameter of inhibition of 17.5 mm (mm) against *C. albicans*, but against *C. tropicalis*, the diameter of inhibition was 15 mm According to Santoyo et al. (2009), aqueous extracts of A. bisporus were undervalued because they consistently outperformed methanolic extracts in antimicrobial results, despite the fact that some potential antimicrobial compounds were destroyed during extraction; Jain & Choudhary (2012) demonstrated the antioxidant activity of the A. bisporus fungus through the activity of removing free radicals (DPPH), and chemical analysis revealed the presence of biologically active molecules.

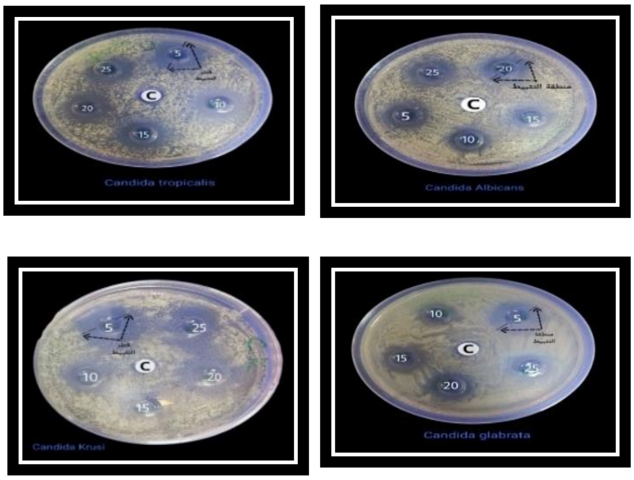


Figure (2) of hot aqueous extract inhibition diameters (1. C. albicans, 2. C. tropicalis, 3. Crusia krusi and 4. Crusia glabrata) and with different concentrations: - (5, 10, 15, 20, 25) mg / ml

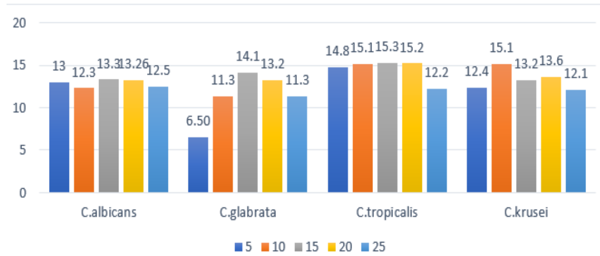


Figure (3): Effect of alcoholic extract (ethanol) on Candida species with concentrations (5-10-15-20-25) mg/ml and an incubation period of 48 hour

The results showed t hat the ethanol extract had the highest percentage of inhibition at a concentration of 15 mg/ml, where the diameter of inhibition for C. tropicalis was 14.4 mm, as it was for C. krusi, C. glabrata, and C. albicans. It gave a diameter of inhibition of 13.8, 13.6, and 13.1 mm, which agrees with (Manuscript, n.d.), where sub-inhibitory concentrations of ethanol extracts showed a decrease in virulence factors and inhibition zones.

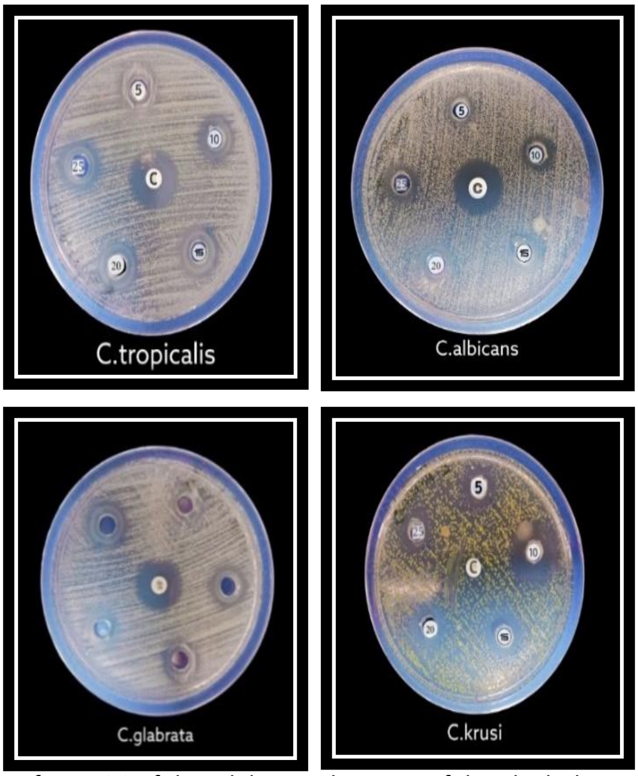
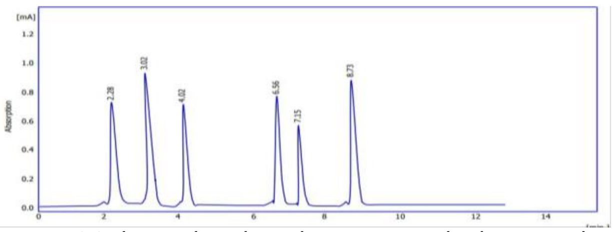


figure (4) of the inhibition diameter of the alcoholic extract (ethanol) 1. C. albaican, 2. C. tropicalis, 3. C. krusi, and 4. C. glabrata at different concentrations: (5, 10, 15, 20, 25)

The results of the high-performance liquid chromatography (HPLC) analysis showed the presence of many phenolic compounds, such as apigenin, ferulic acid, gallic acid, hydrobenzoic acid, qurcetine, and rutin, in the following amounts:

Table (1) Percentages of phenolic compounds in the alcoholic extract (ethanol) and the aqueous extract in units (ppm)

Hot Aqueous	Alcoholic	Name (ppm)	NO.
25.9	32.6	Apigenin	1
40.1	45.9	Ferulic acid	2
75.9	88.9	Gallic acid	3
18.9	24.6	Hydro benzoic acid	4
65.9	74.5	Qurcetine	5
69.8	80.9	Rutin	6



Figure(5) shows the phenolic compounds shown in the HPLC analysis of the alcoholic extract (ethanol).

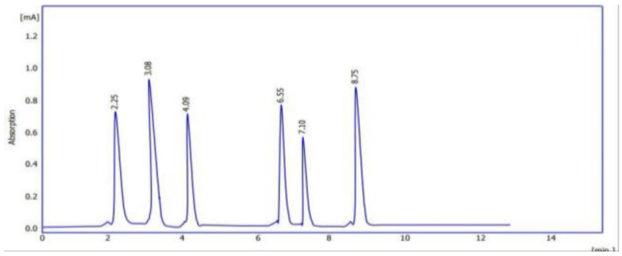


Figure (6) shows the phenolic compounds shown in the HPLC analysis of the hot aqueous extract

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