The antifungal activity of aqueous and alcoholic extract of mushroom (Agaricus bisporus) against Aspergillus flavus

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Abstract, Antifungal activity of aqueous and ethanol extracts of Agaricus *bisporus* were computed in vitro against *Aspergillus flavus* following poison food technique using different concentration (5,10,15,20,25,30 mg/ml) for aqueous extract and (2, 4, 6, 8, 10, 16 mg/ml) for ethanol extract. the maximum effect of ethanolic extract against *Aspergillus flavus* growth was achieved at concentration 10 mg/ml, the growth rate reach 1.25 cm while the maximum effect of ethanolic extract achieved at concentration 16 mg/ml, the growth rate reaches 2.5 cm.

Keywords: mushroom, antifungal activity, antifugal, *A.flavus*

1. Introduction

Agaricus bisporus, is an edible basidiomycete fungus and the most widely cultivated mushrooms in the world, best known as table mushroom. the most familiar form bears white cap, brown gills as well as stalk and flesh (Jagadish et al., 2009).

Plants, mushrooms and other natural sources represent the origin of biologically active compounds, Mushrooms to live in their usual surroundings need antibacterial and antifungal compounds, therefore many mushroom species can act as good source for antifungal compounds which could be beneficial for humans (Yamaç and Bilgili, 2006). Pathogenic fungi result in great loss in humans, crops, farm animals, and other organisms. Moreover, fungal invasion in agriculture leads to a severe reduction in the yield quality of crops and bears massive economic losses. Introducing genes into crops encoding antifungal proteins to encourage their resistance against fungal pathogens may supply ways to treat the problem, (Chu et al, 2005 and Wong et al, 2010). There are two main reasons behind the raises of global interests from the scientific and clinical community to use of mushrooms with potential therapeutic properties First, mushrooms proved their efficiency against many diseases and serious metabolic disorders such as cancer. Secondly, there are many origin sources for fungal bioactive metabolites (wild, mycelial biomass, cultivated fruiting bodies and supernatant of submerged cultured) (Poucheret et al, 2006). The present work detailed and evaluate the effect of aqueous and alcoholic extract of edible mushroom (*Agaricus biosporus*) against pathogenic fungus *Aspergillus flavus*, the random use of drugs by means of commercial antimicrobial it results in drug resistance into human pathogenic microorganisms which encourage the scientists to search new substance act as active antimicrobial from different sources (Karaman et al, 2003).

2. Material and methods

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2.1. Tested organisms:

A. The mushroom (*Agaricus bisporus*) used in this study was obtained from local markets in Mosul, Nineveh province, Iraq
B. *Aspergillus flavus* strain isolated from fruits and identified according to the color and general morphology using light microscope (Pitt and Hocking, 2009*).*

2.2. Identification of *Aspergillus flavus*

Aspergillus flavus was identified to specie level by inoculating on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), and 25 % Glycerol Nitrate Agar (G25%N) at 25 and 37°C for 7 days, and identified by using the identification keys from (Pitt and Hocking, 2009*).*

2.3. Preparation of *Agaricus bisporus* crude extracts:

To prepare the mushroom aqueous extract fresh fruiting body was washed twice and rinsed with distilled water, and dried in shade condition, and grounded into powder by a grinder, 5gm of powder was soaked in 50 ml of water and ethanol (96%) and incubated for 36 hr at room temperature. Whatman filter no 4 was used to filtrate the extracts, water bath at 100°C for water and at 78°C for ethanol used to evaporate the extra solvent from the filtrate, the differences in weights before and after evaporation were calculated (Ejikeme and Henrietta, 2010) and (Jain and Choudhary, 2012). The extracts stored at 4°C in a sterile container for further use.

2.4. Screening the antifungal activity of *Agaricus bisporus*:

Screening the antifungal activity of mushroom (*Agaricus bisporus*) was done according to poison food technique (Alves et al, 2012) 3 ml of each extract incorporated in the culture medium Potato Dextrose Agar (PDA) at concentration (5,10,15,20,25.30 mg/ml) for aqueous extract and (2,4,6,8,10,16 mg/ml) for alcoholic extract.

To determine the antifungal activity of aqueous and alcoholic extracts of *Agaricus bisporus* the colony growth rate in the poisoned plate and non-poisoned plate(control) was compared, the colony growth rate was measured by considering the two orthogonal mean diameters of the colony.

Fig. 1: *Aspergillus flavus* isolate on Potato Dextrose Agar Media

3. Result and Discussion

3.1. *Aspergillus flavus* identification:

Aspergillus flavus was identified according to agricultural and microscopic features and ensured by inoculating on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), and 25 % Glycerol Nitrate Agar (G25%N) at 25 and 37°C for 7 days, and identified by using the identification keys from (Pitt and Hocking, 2009*).*

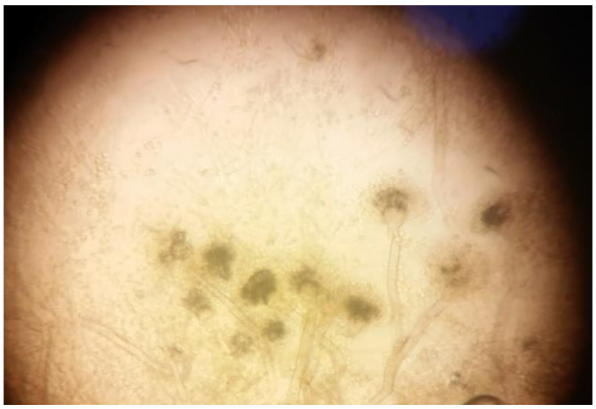

Fig. 2: *Aspergillus flavus* isolate under a microscope (40x)

3.2. Antifungal activity:

The results of the antifungal response shown by the aqueous and alcoholic extract of *Agaricus bisporus* are summarized in tables (1&2) and figures 3,4 and 5.

Table (1): The effect of aqueous extract of *Agaricus bisporus* on the growth of *Aspergillus flavus*

Sr.no	**The concentration of aqueous extract (%)**	**Colony growth rate (cm)**
1	Cont.	8.5
2	5	2.75
3	10	1.25
4	15	2.5
5	20	2.25
6	25	2.25
7	30	2.5

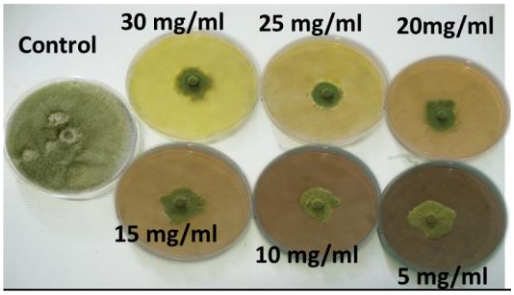

Fig. 3: The effect of aqueous extract of *Agaricus bisporus* on the growth of *Aspergillus flavus*

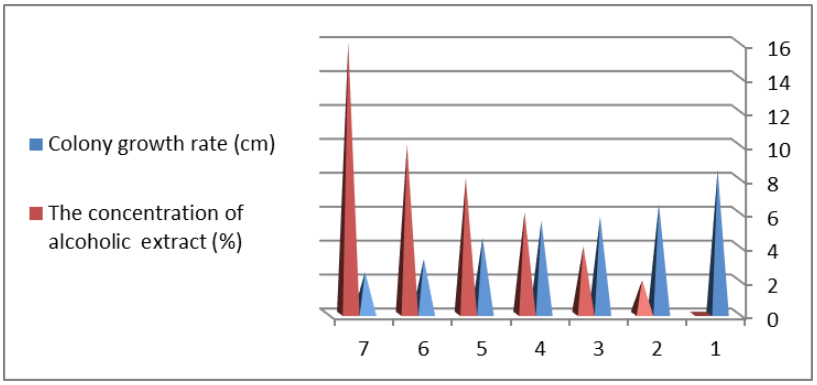

Fig. 4: The effect of aqueous extract of *Agaricus bisporus* on the growth of *Aspergillus flavus*

Table (2): The effect of alcoholic extract of *Agaricus bisporus* on the growth of *Aspergillus flavus*

Sr.no	The concentration of alcoholic extract (%)	Colony growth rate (cm)
1	Cont.	8.5
2	2	6.5
3	4	5.75
4	6	5.5
5	8	4.5
6	10	3.25
7	16	2.5

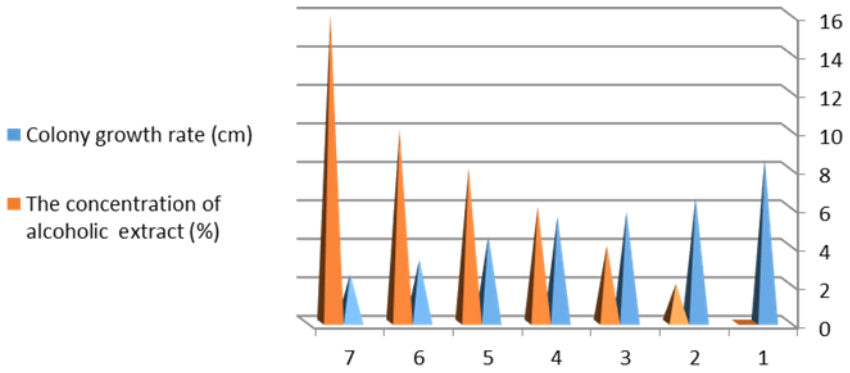

Fig (5) : The effect of alcoholic extract of *Agaricus bisporus* on the growth of *Aspergillus flavus*

There was an obvious activity of aqueous and alcoholic extract on the growth of *A.flavus*, the maximum effect of aqueous extract was achieved at concentration 10 mg/ml, the growth rate reached 1.25 cm while the maximum effect of alcoholic extract archived at concentration 16 mg/ml, the growth rate reach 2.5 cm. Mushrooms are rich with proteins and compounds which show antifungal, antibacterial and anti-viral activities, polypeptide alveolarin and peptide eryngin have extremely antifungal activity (Turkoglu et. Al., 2006, Wang and Ng, 2000, Wang et.al., 2004, Solak et.al., 2006, Cohen et.al., 2002, Bender et.al., 2001). There are many studies refried to the antimicrobial activity of mushroom extracts against many microorganisms (Barros et.al., 2007 and Demirhan et.al., 2007). Kumar and Yadav, 2014 report that the ethanolic extract of *Agaricus bisporus* exhibited antifungal activity against *A.flavus*. Öztürk et al. 2011 reported antifungal activity of methanolic extracts of A. *bisporus*, *A. essettei* and *A. bitorquis* against *Candida albicans* while Barros et al. 2008 could not found the activity of *Agaricus bisporus* against *Candida albicans*.

4. Conclusion:

It was concluded from the results of the present work, the aqueous and ethanol extract of *Agaricus bisporus* showed good activity against *A.flavus, so that Agaricus bisporus* is useful for health and could be used as a natural source of the antifungal agent in enlargement new drug for fungal infections instead of the use of commercial antifungal drugs which result in drug resistance.

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