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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA INFECTION AND TREATMENT BY ACTIVE SUBSTANCES ISOLATED FROM *AGARICUS BISPORUS* FUNGI

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Abstract

The present study aimed to evaluate the antibacterial activity (phenols) of *Agaricus bisporus* mushrooms and their effect on isolated human pathogen bacteria. The study mediating 200 samples were collected between July and November 2019, where the number of positive samples was 102, while the number of negative samples was 98, using sterile transport media swabs from various Patients in hospitals from three hospitals in Baghdad. six species were isolated from bacteria and the most common genus or species of bacteria isolated were *Escherichia coli* 26 samples *and* the occurrence ratio of 23 samples for *Staphlococcus*, and 20 sample for *Acinetobacter* and the occurrence ratio of 19 sample for *Klebsiella* and the occurrence ratio of 11 sample for *Pseudomones* and the occurrence ratio of 3 samples for *Proteus*. All bacteria isolates were identified depending on the morphological and microscopic examinations. Three concentration (25, 50, and 100 mg / ml) was prepared from crude extract phenols, The results obtained for phenol extract indicate that the highest level of inhibition zone was detected at a concentration of 100 mg / ml where the inhibition regions are (2.47 ± 0.29) cm on bacteria *Acinetobacter*.

Keywords: pathogenic bacteria, *Agaricus bisporus*, fungi

Introduction

Agaricus bisporus extract are rich in bioactive compounds, such as phenols, terpenoids and alkaloids which is the major active components in the mushroom .these compounds with anti bacterial activity can be explored and use for the control and reduce of bacterial diseases. However, *Agaricus bisporus* Mushroom body extract is use in this study to Treatment of human pathogenic bacteria with effective mushroom-derived substances (Sadiq *et al*., 2008). *A. bisporus* is a litter degrading basidiomycete commonly found in humic-rich habitats that are useful as a model organism and are widely cultivated for the food industry. Due to its ecological niche, it produces a variety of enzymes for the detoxification and degradation of the humidified plant litter (Gonaus *et al*., 2016).

Materials and Methods

Preparation of mushroom extracts

Crude extract

The fruiting bodies of the mushrooms are dried in the shade. Dry fruiting bodies are cut into small pieces using scalpels and crushed using a pistil and a motor. And 50.0 g of mushroom powder had been stored in the flasks of Erlenmeyer, where 95 per cent of methanol had been put. The flasks were covered with aluminum foil and were allowed to stand for 7 days for extraction. The solution was filtered through Whatman filter paper No. 1 and the filtrate was concentrated in a rotary evaporator. Methanol has been evaporated and the extract has been collected and dried (Bruna *et al*., 2016).

Phenols

Mushroom *A. bisporus* Samples have been crushed and taken (1 g) of powder and placed into ethanol (10 ml; 80%). Test mixtures are stored at room temperature in the water bath (20 min) and then centrifuged (3500 rpm; 15 minutes) and filtered by filter paper (Bennett *et al*., 2004).

Phenolic indicators

Ferric chloride and Potassium ferric cyanide reagent

It was used to detect general phenols. Prepared by taking 2 equivalent amounts of 1 percent ferric chloride and 1 percent potassium ferric cyanide aqueous solution. Blue-green color was shown indicating that the test was positive (Harborne, 1984).

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Results and Discussions

Isolation and diagnosis of human pathogen bacteria from people with diseases

Different colonies of bacteria were observed on plate at 37°C for 24-48 hour and appeared with different morphological features. Bacterial colonies vary in shape, size (measured in diameter), odor, color (pigmented), texture, and degree of media adherence (pitting and crusting). Different bacteria have various colony morphologies, including rhomboidal morphologies (e.g. *Pseudomonas* spp.), large mucoid colonies (e.g. *Klebsiella* spp.), swarming colonies (e.g. *Proteus* spp.). Colonies may be mucoid (M colonies), smooth (S colonies) and (R colonies) dry (Tortora *et al*., 2001).

Pseudomonas aeruginosa

They are gram-negative bacteria, aerobic, bacillus (Ryan and Ray, 2004). It has a characteristic grape-like smell of aminoacetophenone. It is a pure aerobic with a temperature range of 5-42 °C. Most other *Pseudomonads* do not grow at 42 °C. *Pseudomonas aeruginosa* is a rod-shaped, mobile organism (polar flagella) that produces water-soluble pigments that disperse through the medium. The characteristic blue-green appearance of colonized / infected pus or organism culture is due to the mixture of pyocyanine (blue) and pyoverdin (fluorescein, yellow). The development of blue-green pigments is indicative of *P. aeruginosa* which may be produce at least six colonial species after aerobic incubation of nutrient agar for 24 hours at 37 °C. the most common type of colonies, which are broad, short, oval, convex and rough, often surrounded by serrated growth (Pitt and Simpson, 2006).

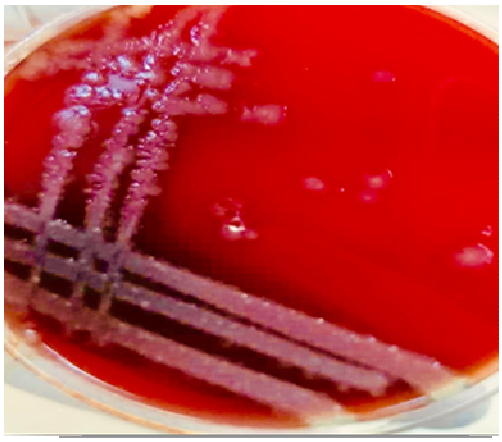


Fig. 1 : *Pseudomonas* grows on MacConkey agar at 37 °C after one day

Escherichia coli

Escherichia coli is a rod-shaped appearance that has been arranged in single or pair and has a white color colony growth on nutrient agar. Each bacterium is approximately 0.5 µm in width by 2 µm in length. *E. coli* is a Gram-negative bacteria. *E. coli* cells are stained Gram negative because they have a thin cell wall with only 1 to 2 layers of peptidoglycan. *E. coli* is an optional anaerobic, which means that it does not require oxygen, but grows better in the presence of oxygen (Cummings and Macfarlane, 1997).

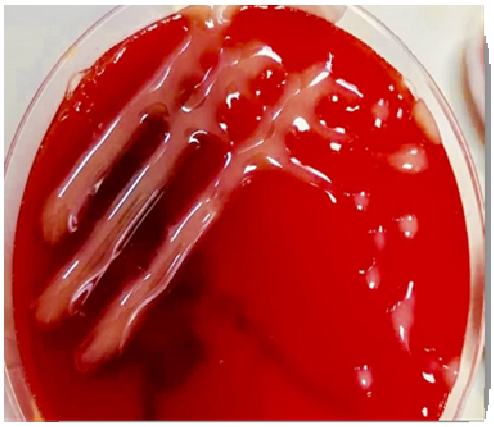


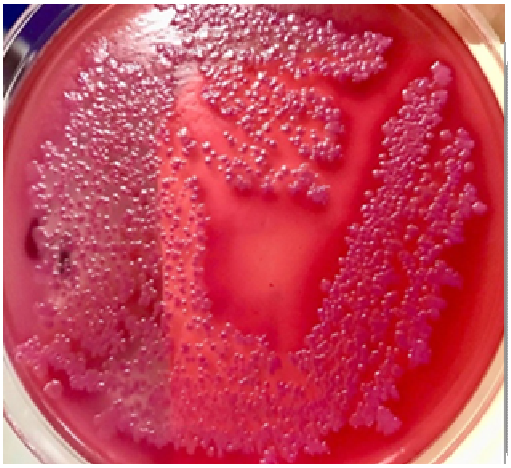
Fig. 2 : *Escherichia coli* grows on MacConkey agar at 37 °C after one day

Proteus

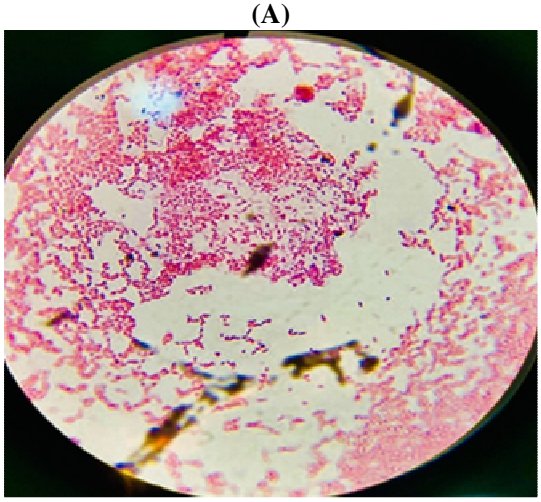
Proteus are dimorphic bacteria which, in liquid media, are motile, peritrichously flagellated short rods (1.0 to 2.0 mm in length with 6-10 flagella) such bacteria are called swimming cells. However, when transferred to solid media, these short rods transform into elongated (20-80 mm in length), hyperflagellated, multinucleated, non-septated swarmer cells. The latter migrate from the inoculation site as long as the population of swarmer cells is reduced on solid surfaces. Then the process of restructuring takes place. In this period of swarming growth, long rods disintegrate into short bacteria. The process of differentiation and dedifferentiation of the Proteus bacteria is cyclic. It results in the formation of characteristic rings of bacterial growth on the agar plate (Morgenstein *et al*., 2010) and has a yellow color colony growth on nutrient agar.

Klebsiella

Klebsiella is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, enteric, rod shaped bacterium present in the normal intestinal flora. It is urea-positive (blue slope colour), metabolizes glucose with gas production (bubbles under a piece of glass-in detail left down side of each plate) and is lactose-positive (but its Endo colonies still remain very pale on agar) (Trivedi *et al*., 2015).



(A)



(B)

Fig. 4 : (A) *Klebsiella* grows on MacConkey agar at 37 °C after one day. (B) *Klebsiella* microscopic feature

Staphylococcus aureus

Staphylococcus aureus with 5 percent de-fibrinated sheep blood (Bio-Rad) on Columbia agar. Individual agar colonies are round, convex and 1-4 mm in diameter and have a straight border. Staphylococcus aureus colonies are often surrounded on blood-agar plates by areas of visible beta-hemolysis. Staphylococci in cluster, chains, and singles arrangements can saw in microscope (Mamza *et al*., 2016).

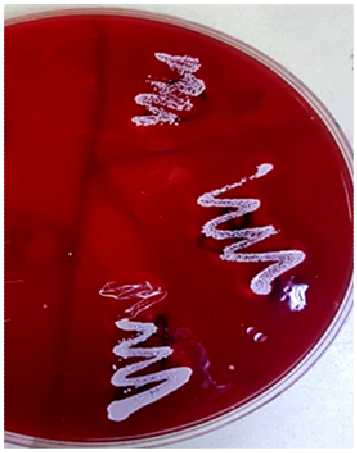


Fig. 5 : *Staphylococcus* grows on MacConkey agar at 37 °C after one day.

Acinetobacter

Gram-negative strains, non-fermented, coccobacilli bacteria, Non-motile and the utilization of many substrates. Grew well on the usual culture mediums and produced colonies of 2-3 mm in diameter at 18-24 hours. The colonies were comparable to enterobacteria. They produced a pale yellow to white-gray pigment on a solid medium. The colonies weren't pigmented when they grew on blood agar (Constantiniu *et al*., 2004).

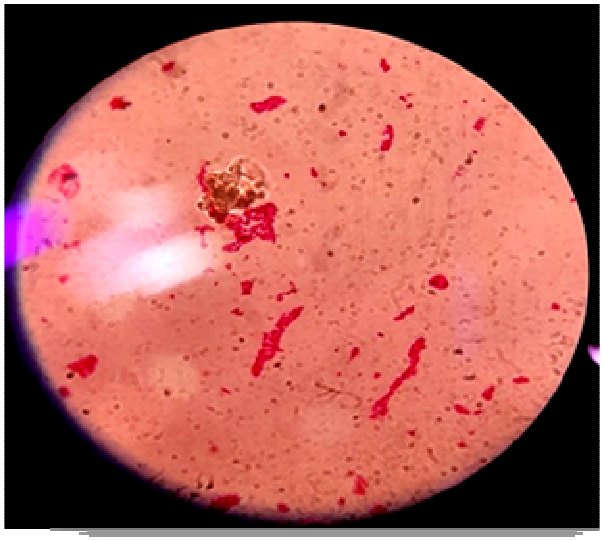
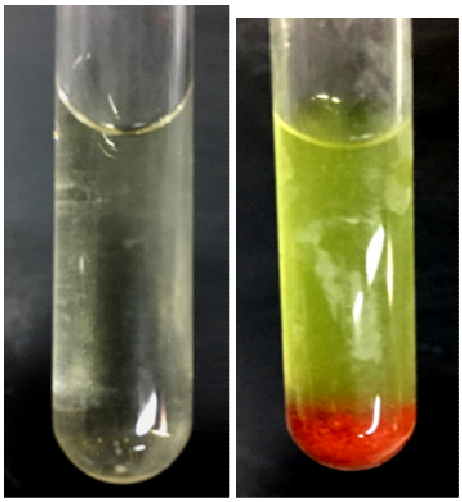


Fig. 6 : *Acinetobacter* microscopic feature

Phenolic indicators

Ferric chloride and Potassium ferric cyanide reagent

When adding the chemical to an extract, we notice a change in color in figure (7) to blue-green color was shown indicating that the test was positive and phenol is present in *Agaricus bisporus* mushroom extract, and these results consistent with the results of (Harborne, 1984).



(A) **(B)**

Fig. 7 : Phenolic indicators: (A) Before adding chemicals (B) After adding chemicals, blue-green color was shown indicating that the test was positive.

Antibacterial activity of *Agaricus bisporus* extracts against *Pseudomonas* bacteria.

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 50 µg/ml of phenol extract

Effect of concentration of mushroom extraction on *pseudomonas*

Extracts	Inhibition zone (cm)				LSD value
	Control	25 µg/ml	50 µg/ml	100 µg/ml	
Phenol	0.667 ± 0.35	1.13 ± 0.06	1.26 ± 0.46	0.933 ± 0.37	1.134 NS

Antibacterial activity of *Agaricus bisporus* extracts against *E. coli*

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 50 µg/ml and 100 µg/ml of phenol extract.

Effect of Concentration of mushroom extraction on *E. coli* bacteria

Extracts	Inhibition zone (cm)				LSD value
	Control	25 µg / ml	50 µg / ml	100 µg / ml	
Phenol	0.200 ± 0.00	0.466 ± 0.26	0.800 ± 0.23	0.800 ± 0.20	0.687 NS

Antibacterial activity of *Agaricus bisporus* extracts against *Acinetobacter*

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 100 µg/ml of phenol extract.

Effect of Concentration of mushroom extraction on *Acinetobacter* bacteria// Inhibition zone

Extracts	Inhibition zone (cm)				LSD value
	Control	25	50	100	
Phenol	0.333 ± 0.08 c	1.67 ± 0.16 b	2.33 ± 0.33 ab	2.47 ± 0.29 a	0.783 *

Antibacterial activity of *Agaricus bisporus* extracts against *Proteus*

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 25 µg/ml of phenol extract.

Effect of Concentration of mushroom extraction on *Proteus* bacteria

Extracts	Inhibition zone (cm)				LSD value
	Control	25	50	100	
Phenol	0.00 ± 0.00	0.200 ± 0.20	0.133 ± 0.06	0.00 ± 0.00	0.344 NS

Antibacterial activity of *Agaricus bisporus* extracts against *Klebsiella*

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 50 µg/ml of phenol extract.

Effect of Concentration of mushroom extraction on *Klebsiella*

Extracts	Inhibition zone (cm)				LSD value
	Control	25 µg/ml	50 µg/ml	100 µg/ml	
Phenol	0.00 ± 0.00 c	0.266 ± 0.12bc	1.30 ± 0.15 a	0.700 ± 0.30 b	0.582 *

Antibacterial activity of *Agaricus bisporus* extracts against *Staphylococcus*

The obtained results indicate that the highest level of inhibition zone was detected at the concentration of 25 µg/ml of phenol extract.

Effect of concentration of mushroom extraction on *Staphlococcus*

Extracts	Inhibition zone (cm)				LSD value
	Control	25 µg/ml	50 µg/ml	100 µg/ml	
Phenol	0.00 ± 0.00 b	1.00 ± 0.00 a	0.366 ± 0.31 ab	0.500 ± 0.28 ab	0.700 *

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