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UTILIZATION OF *AGARICUS BISPORUS* TO INHIBIT THE GROWTH OF SOME MICROORGANISM SPECIES

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Abstract

A number of culture media were prepared from *Silybum marianum* (*Asteraceae* family) and from peel of *Punica granatum* (Lythraceae family). The effect of concentrations of 5, 10, 20, 30 and 40% of these agars on the Mycelia of *Agaricus bisporus* (Agaricaceae family) at the mother culture stage was studied. The obtained result showed that best effect were found at the concentration of 30% of *Silybum marianum* and 5% peel of *Punica granatum*. Qualitative chemical detection of the first culture media extract (Mycelia of *Silybum marianum)* revealed that it contains high glycosides compared with the second culture media extract (peel of *Punica granatum*) in addition to the presence of alkaloids, tannins and flavonoids in both extracts. However, the tannins were found to be less in the first extract and distinguished in the second extract, while the saponin disappeared at the time of detection in both extracts. The study also showed that the water extract of *Silybum marianum* (30%) was higher inhibition ratio compared to the water extract of peel of *Punica granatum* (5%) of the species of bacteria *Esherichia coli, Bacillus cereus and Staphylococcus aureus.*

Keywords **:** *Punica granatum, Agaricus bisporus, Silybum marianum,* flavonoids.

Introduction

Culture media are consider as appropriate environments where the microorganisms are grown due to its content of nutrient requirements for growth such as nitrogen, carbon, phosphorus, sulfur and other elements (Kazanji, 2017). In the second half of the 19th century, interest was evident in these culture media and the researchers were still working on the creation of new types and the development of old types to improve their natural or industrial efficiency (Elias, 2008). The developments in these agars showed a positive reflection on the evolution of life sciences (Difco Manna, 1999). Currently, some of the natural culture media have been used to develop some of the fungus species of medicinal importance, such as *Agaricus bisporus* (Rashid *et al*., 2018). These culture media were characterized by being cheap and inexpensive in preparation as well as available and manufactured from plant materials and animal material. Moreover, some of them are of medical importance, have enabled researchers to think about the production of improved fungus with their nutritional and medical value, taking advantage of the ability of some edible fungi to absorb substances from their developing agar to the tissues of the same fungi (Dhar and Shrivastava, 2012). Therefore, the present study is to investigate the possibility of using some of the plants known to be of medical importance, including the plant *Silybum marianum* and *Punica granatum* as a basis in the preparation of culture media to develop mycelia of *Agaricus bisporus* at the stage of mother culture and study the extract effect of those mycelia on some species of gram positive and negative bacteria. As these plants are characterized by their extensive use in the modern medical field.

Materials and Methods

Preparation of the Plant Culture Media

The new stems and leaves of the plant *Silybum marianum* were used in the preparation of the culture media according to the method of Abdelkader *et al*. (2012). The stems and leaves were washed to remove the dust and dried, chopped and mixed well in a mixer and then the following treatments were prepared (5, 10, 20, 30 and 40 % by adding 6.25, 12.5, 25.0, 37.5 and 50 g/L of distilled water respectively). The solution was then filtered with piece of cloth and stored in the refrigerator until use. As for peel of *Punica granatum*, it was prepared by washing, drying and grinding the peel of *Punica granatum* and then amount of 5, 10, 20, 30 and 40 g/L were weighed to prepare concentrations of 5, 10, 20, 30 and 40% respectively. Then 18 g/L of the agar was added for each treatment and all treatments were sterilized at a temperature of 121 ºC for 15 minutes (Macfaddin, 2000).

Preparation of manufactured culture media: It was prepared according to the instructions of the equipped company (HIMDIA):

- Potato Dextrose agar (PDA): dissolve 39.5 g/L of distilled water (control)
- Nutrient agar (NA): dissolve 28 g/L of distilled water (to estimate the diameter of the inhibition zone of *A. bisporus* extract).

Nutrient broth (NB): Prepare to dissolve 18 g/L of distilled water. In order to activate the isolates of the used bacteria.

After that all the plant culture media and all the prepared media were sterilized at 121 ° C for 15 min and 15 lb / 2 pressure (MacFaddin, 2000)

Experimental microorganism types: *Agaricus bisporus* (brown strain) was obtained from the fungi production unit of the Medical Plant Production Unit of the Faculty of Agricultural Engineering Sciences - University of Baghdad. The isolates of *Esherichia coli, Bacillus cereus* and *Staphylococcus aureus* were obtained from the Graduate Laboratory of the same faculty. The plant culture media and PDA were prepared and transferred to laboratory dishes with dimension of 90-mm and it was vaccinated with a disk from the end of the fungi *A bisporus* colony, which was activated by the Cork borer on the PDA medium. The disc was placed in the center of the dish and the dishes were closed with paraffin and incubated at 25 ° C and the growth rates of the developing colony was measured daily until growth is complete. Growth rates are calculated according to the following equation:

$$A = \frac{B}{C}$$

A: growth velocity, B: diameter of the developing colony on which the fungus haze (90 mm)

C: the required time for growth of the mycelia on the same dish.

Preparation method of *Agaricus bisporus* extracts: The dishes of the mentioned fungus which growing on plant culture media (30% *S. marianum* and 5% peel of *P. granatum*) were scraped as well as those growing on the PDA medium for comparison and under the ideal conditions specified in this study. Transfer 1 g of fungal mycelium to 10 mL of distilled water in a test tube then, mixed and homogenized using an electric mixer. The cells were fragmented on the ultrasound device at 20 Hz for 8 minutes, then centrifuged at 3500 rpm and filtered with filter paper 0.4. Thereafter filtered through a membrane filter with 0.22 µm openings to sterilize microbial contaminants.

Activation of bacteria using NB: *E. coli, Staphylococcus aureus* and *Bacillus cereus* were activated by transferring the Loop to the NB medium from the bacterial farm and incubated at 37 °C for 18 hours (Chung and Drake, 2006).

Effect of fungal extract in bacteria: The N.A media was prepared and pour in dishes and let it solidify completely. The activated bacteria were streaked on both sides individually and thickly on the surface of the medium. A number of holes were drilled in each dish using a cork borer and each 5 µl of each extract was transferred to each hole and then incubated dishes at 37 °C for 48 h and measured the diameter of the halo formed around the hole in the dish (Kazanji, 2017).

Specific chemical detection of active compounds in the extract *A. bisporus*: The methods of Harborne, (1973) were followed to detect for tannins, glycosides, flavonoids, saponins, and alkaloids in the current research:

Detection of tannins: Amount of 2 g of mycelia was boiled in 10 ml of distilled water, and the solution was filtered and left to be cooled. After that divided into two parts where 1% of lead acetate solution was added to the first part to indicate the presence of tannins by forming the gelatinous staining, while 1% of ferric chloride solution was added the second part and when the solution was greenish blue, indicating the presence of tannins.

Detection of glycosides: Two equal parts of fehling reagent (A and B) were mixed with the extract and heated in a boiling water bath for 10 minutes. The presence of the glycosides was indicated by the presence of polysaccharides with a red-colored deposit.

Detection of alkaloids: Amount of 2 g of mycelia was boiled in 10 ml of hydrochloric acid 4% and the solution was filtered after cooling, and testing 0.5 mL of leachate in a watch glass bottle with each of the following reagents:

Meyer reagent: Observing a white deposit indicates the presence of alkaloids.

Dragendorff's reagent: Watch an orange deposit indicating the presence of alkaloids.

Detection of flavonoids: Solution (A) was prepared by dissolving 2 g of mycelia in 1 ml of 95% ethyl alcohol and then the solution was filtered while solution B was prepared by adding 2 mL of ethyl alcohol (50%) to 10 ml of potassium hydroxide solution (50%). A yellow ring indicates the presence of flavonoids.

Detection of saponins: Amount of 2 ml of cold distilled water was added to 2 ml of water extract with continuous shaking for 1 minute, the presence of saponin was indicated with bubbles.

Results and Discussion

Qualitative chemical detection of active ingredients in the fungus extract of *A. bisporus*: Table 1 showed the results of specific chemical tests for the detection of the chemical nature and the active ingredients found in the water extract in *A. bisporus* extracts grown on the medium of the *Silybum marianum* at a concentration of 30% and the peel of *Punica granatum* at a concentration of 5%. It was found that the presence of alkaloids was clearly noted, as well as tannins and flavonoids in both extracts, but the tannins was less visible in the first extract and distinct in the second while the saponin was disappeared when detected in the extracts. The results of the present study are consistent with the findings of Yuanzheng and Shin, (2016) who detected the presence of glycosides in the mycelia and fruit bodies of some edible fungi, such as Sinigrin, Glucoberin, Glucocleomin, Glucocapparin, and Glucobrassicin, which play an essential role in increasing the growth speed and density of mycelia of these fungi. Glycosides are one of the important storage sources of sugar storage in the regulation of osmotic pressure within the cell of the organism (Alani, 1998). Flavonoids are found freely or in the form of glycoside derivatives, which are a general inhibitor of cancer cells and antibiotic resistance mechanisms for a wide range of pathogenic bacteria due to their ability to dissolve the cellular proteins of bacteria and thus break down the cell membrane of the bacteria (Hamid, 2018). Which was reflected positively on the detection of flavonoids in the extract of the developing mycelia on that medium.

Table 1 : Qualitative chemical detection of active ingredients in the extract of *A. bisporus* fungus.

Compounds	Used detector	Detection index	Detection result of the extract of developing mycelia on culture media		
			PDA extract	*S. marianum* (30%)	*Peel of P. granatum* (5%)
Tannins	Lead acetate 1%	Bleaching white precipitate	+	++	+++
	Ferric chloride 1%	The appearance of a greenish blue color	+	+	+++
Glycosides	Fehling	Red precipitate	+	+++	+
Alkaloids	Mayer	White precipitate	+	++	++
	Dragendorff's reagent	Orange precipitate	+	++	++
Flavonoids	Ethyl alcohol 95% + KOH	A yellow-ring appears	+	++	++
Saponins	Cooled distilled water	Air bubbles appears	-	-	-

Effect of different concentrations of *Silybum marianum* and peel of *Punica granatum* on the rate of growth of the fungus *A .bisporus* at the mother culture stage: Table (2) shows the effect of the different concentrations of the natural factors of the transactions, which ranged from 5 to 40% in the growth rate of *A. bisporus* fungus each day and thus the rapid growth of these mycelia on the dish. The average duration of growth for each day was inversely affected with the concentration of the *Silybum marianum* treatments to a concentration of 30%. After that, the average growth period of fungus increased to 16 days for treatment of *Silybum marianum* 40%. The mean growth durations were 5, 10, 20 and 30% for *Silybum marianum* for days 12, 11, 10, 7 respectively. Which recorded a growth rate 7.50, 8.18, 9.00, 12.85 mm/day while the growth rate of these mycelia at concentration of 40% was slowed significantly to 5.29 mm/day. Additionally, table (2) also shows that concentrations of peel of *Punica granatum* have been directly proportional to the average growth duration of each day. The concentrations of peel of *Punica granatum* (5, 10, 20, 30 and 40%) showed an average growth period of 8, 9, 13, 15 and 18 days respectively, and at a growth rate of 11.25, 10, 6.92, 6.00 and 5 respectively, for the same fungi. Hence, the treatments *Silybum marianum* (30%) and peel of *Punica granatum* (5%) were adopted in the next stages of research because they achieved the highest growth rate and less days. This was in comparison with the standard growth rate of the edible fungus PDA, which achieved a growth rate of 6.42 mm/day with an average growth period of 14 days.

The rapid growth rate of *A. bisporus*, recorded by the *S. marianum* (30%) and peel of *P. granatum* (5%) reflects the content of these cultures of the material necessary for the growth of the fungus and the standard ratios, especially the ratio of nitrogen to carbon, which plays a key role in the efficiency and activity of edible fungi in general. The low growth rate in other treatments showed difficulties to adapt to these culture media, thus showed the speed of growth and the length of time needed in days. It is possible that high concentration of N inhibited the growth of mycelium of *A. bisporus*. Amran *et al*. (2011) found that the fungus ability to use different nitrogen sources has a significant role in stimulating the production of enzymes specialized in the analysis of complex substances in the nutrition medium and convert them to easily digested substances but within a certain range, if they increase, the growth process is discouraged and sometimes stopped. The different rates of growth velocity in different culture media may be due to the different components of organic compounds and minerals. Several studies have shown that the *S. marianum* contains an important strategic reservoir that makes it a leading source of nutritious and effective nutrient in the development of the fungi which has a positive effect on its growth on the medium made of it. The most important of these materials is silymarin, it is also excellent as antibacterial and it has a positive effect on the liver and rebuild it again, as well as antioxidant effects and cancer tumors (Bajwa *et al*., 2016). Moreover, peel of *P. granatum* is rich in vitamins and mineral salts that help to give vitality to the developing mycelium, such as potassium, sodium, calcium, phosphorus and magnesium, as well as amino acids and unsaturated fatty acids such as flavonoids which help to increase the growth rates of mycelia of the fungi under the study (Akiyama *et al*., 2011).

Several researchers have studied the effect of different concentrations of the materials used in preparing culture media for the development of a group of fungus in the production of edible fungus. Muslat, (2007) found that the best concentration of *Ceratophyllum demersum* in growth rate of *Pleurotus ostreatus*, *Fusarium moniliforme* and *Trichoderma harzianum* average was 100 g/L with a growth rate of 1.125 cm/day and three concentrations of 150, 100 and 50 g/L. Moreover, it was detected that moisturized medium (60 g/L) was higher than that of the nutritious agar (NA) for the purpose of the development of bacteria *Staphylococcus aureus,* which reflex its high content of protein and carbohydrates as well as elements metal that important to feeding and growth of bacteria (Muhsin 2008).

Table 2 : Effect of different concentrations of *Silybum marianum* and peel of *Punica granatum* on the rate of growth of the fungus *A. bisporus* at the mother culture stage

The growth rate of mycelia on the dish (mm/day)	Average duration of growth (day)	The type of the media
6.42	14	PDA
7.50	12	*S. marianum 5%*
8.18	11	*S. marianum* 1o%
9.00	10	*S. marianum* 20%
12.85	7	*S. marianum 30%*
5.29	16	*S. marianum 40%*
11.25	8	Peel of *P. granatum 5%*
10.0	9	Peel of *P. granatum 1o%*
6.92	13	Peel of *P. granatum 20%*
6.00	15	Peel of *P. granatum 30%*
5	18	Peel of *P. granatum 40%*

Effect the mycelium of *A. bisporus* extracts on the types of bacteria under study: The objective of this part of the study was to identify the possibility of the transfer of some active substances from the components of the media to the mycelia of *A. bisporus*. The mycelia of fungus were collected and dissected by ultrasound and its components were extracted with water and studied the effect of these extracts on microorganisms (Table3). It is noted that the water extract of *A. bisporus* growing on 30% of *S. marianum* and 5% of peel of *P. granatum* inhibited the growth of the three types of bacteria (*E. coli, Bacillus cereus and Staph. aureus*). Although with varying degrees, the diameter of the inhibition area for the *E.coli* bacteria was 4.6 and 0.3 mm and for *Bacillus cereus* was 8.1 and 5.9 mm while it was 7.9 and 6.7 mm for *Staph. aureus* bacteria for both types of medium respectively. However, the effect of the water extract on the fungus of the PDA medium was limited. Additionally, the effect of the extracts on the positive bacteria was greater than the negative bacteria (represented in this experiment with *E. coli*). This indicates that some of the active ingredients found in *S. marianum* and peel of *P. granatum* were transferred to the fungus cells during growth in these media. The active ingredients included tannins that inhibit microorganisms, which are known to absorb water, making them unavailable to the developing bacteria in the medium where they are found (Muhammad, 1981) resulted in deforms and loses the basic function of the cell (Schultz *et al*., 1992), as well as containing similar-type components in the fungus bodies. The difference in susceptibility to this extracts may be due to the cell wall structure and regulate the outer membrane of negative gram bacteria and gram positive bacteria, as shown

by some studies that the plant extract affects the gram positive bacteria more than gram negative because of the differences in the outer layers of the cell wall of gram positive and negative bacteria, as the negative bacteria contain exceptional external membranes does not exist in gram positive bacteria, and antibacterial agents easily damage the cell wall leading to cytoplasm out of the cells and coagulation, resulting in cell death. (Kazanji, 2017).

Kalyoncu *et al*. (2010) have found that the extract of ethyl alcohol for *Pleurotus eryngii* can inhibit *Candida albicans* as well as *Fusarium velutipes* fungi have a resistance against *Fusarium culmorum* and *Rhizoctonia cerealis*. In addition, Yim *et al*. (2010) investigated the effect of phenolic materials extracted from *Pleurotus ostreatus* in inhibiting the growth of seven types of bacteria and two types of yeasts, and found that there was a difference in the inhibition rates according to the concentration of extracted phenolic material in the mycelia and fruit bodies that affected by the type of environment and the conditions surrounding the development process. Al-Wondawi (2011) found that the fungus extract of *Volvariella volvacea* showed an inhibitory effect on fungal pathogens *Fusarium oxysporum*, *Rhizoctonia solani*, and *Alternaria solani* by the effect of alkaloid and phenolic substances found in fruit bodies. Furthermore, Mondal *et al.* (2013) demonstrated that the fungi *Pleurotus ostreatus* and *Pleurotus sajor caju* contain the highest level of phenolic compounds and flavonoids with inhibitory activity against certain fungal pathogens such as *Rhizopus stolonifer, Aspergillus flavus, Aspergillus candidus* and *Penicillium patulum* compared to others. Erjavec *et al*. (2012) notes that *Pleurotus eryngii* contains Eryngin protein which has an inhibitory effect against fungi *Fusarium oxysporum* and *Fusarium velutipes.*

Table 3 : The effect of *A. bisporus* extract on some microorganisms development

Type of culture media	The rate of inhibition diameter/mm		
	E.coli	***Bacillus cereus***	***Staph. aureus***
PDA	0	2.5	2.8
S. marianum 30%	4.2	8.1	7.9
P. granatum 5%	0.3	1.6	6.7

References

Abdelkader, Z.M. and others. (2014). The yield and potentiality of the oyster mushroom under the influence of various nitrogen sources.

Abdel-Kadhim; Lamia, S.M.; Sereen, S. and Ahmed, S.A. (2008). Study of the inhibitory activity of the willow extract in some types of pathogenic bacteria. Iraqi Journal of Life Technologies, 7(1): (38-50). .

Akiyama, H.; Fuji, K.; Yamasaki, O.; Oono, T. and Iwatsuki, K. (2011). Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 48(4): 487-91.

Al-Ani, O.H.J. (1998). Study of the components of the local black bean *Nigella sativa* and the effect of its extracts on some microorganisms. Master Thesis. College of Science. Mustansiriya University.

Al-Wondawi, D.S. (2011). Effect of some environmental and biotic factors on the production of wheat straws *Volvariella volvacea* and the control of the biota contaminated by its biochemistry. Ph.D., Department of Prevention, Faculty of Agriculture, University of Baghdad.

Bajwa, A. (2016). Evaluation of Anti-bacterial Activity of *Silybum marianum* against Pathogenic and Resistant Bacteria, European Journal of Medicinal Plants, 13(4): 1-7.

Chung, H.J.; Bang, W. and Drake, M.A. (2006). Stress Response of *E. coli*. Comprehensive Reviews in Food Science And Food Safety, 5.

Dhar, B.L. and Neeraj, S. (2012). Mushrooms and Environmental Sustainability Mushroom .Research Development and Training Centre (MRDTC), DK Floriculture , Usha Farm Near Bamnoli Bus Stand, Bijwasan, New Delhi-61 Corresponding Author.

Difco Manual of Dehyrated Culture Media and Reagents for Microbiological and Clinical .Laboratory Procedures, 1999. 9th. Ed., Published by Difco Laboratories Inc.,Detroit and Michigan.

Elias, A. (2008). Effect of feeding communities on the production of *Agaricus bisporus* seeds. Master Thesis, Faculty of Agricultural Engineering / Tishreen University.

Erjavec, J.; Kos, J. and Ravnikar, M. (2012). Proteins of higher fungi–from forest to application. Trends in Biotechnology. 30(5): 259-272.

Harborne J.B. (1973). Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. University of Reading.

Kalyoncu, M.; Oskay, M.; Saglam, H. and Tamer, U. (2010). Antimicrobial and antioxidant activities of mycelia of 10 wild Mushroom species. Journal of Medicinal Food, 13(2): 415–419.

Kazanji, M.O.M. (2017). Scientific experiments in microbiology. First Edition. Baghdad University. Faculty of Agriculture.

MacFaddin, J.F. (2000). Biochemical Test for Identification of Medical Bacteria, 3 rd ed. The Willims and Wilkinson Baltimor. USA.

Majid, S.H. (2018). Study the effect of AgNp-supported honey in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates from urinary tract infection. Journal of the Faculty of Basic Education. Volume 24. Issue 100.

Mohamed, Abdel-Adim and El-Reis A.H. (1981). Plant physiology, Part II. Dar Al Kutub Printing Est.

Mondal, T.; Some, R. and Dutta, S. (2013). Studies on antioxidant and antimicrobial properties of some common mushrooms. Journal of Today's Biological Sciences, 2(1): 60- 67.

Rashid, H.M.; Abed, I.A. and Owaid, M.N. (2018). Mycelia growth performance of *Agaricus bisporus* in culture media of composts supplemented with *Sesbania sesban* straw and phosphate rock. Current Research in Environmental & Applied Mycology 8(3): 323–330.

Schultz, J.C.; Hunter, M.D. and Appel, H.M. (1992). Antimicrobial activity of polyphonls mediates plant – herbivore interactions: plant polyphonls synthesis, properties, significance. Plenum press, New York, p.621.

Yim, H.S. Jr; Chye, F.Y.; Tan, C.T.; Ng, Y.C. and Ho, C.W. (2010). Antioxidant Activities and Total Phenolic Content of Aqueous Extract of *Pleurotus ostreatus* (Cultivated Oyster Mushroom) Malays J Nutr. 16(2):281-91.

Yuanzheng, Wu and Hyun-Jae Shin (2016). Cellulose from the fruiting bodies and mycelia of edible mushrooms. Journal of Mushrooms. Mushrooms 2016 December, 14(4): 1-9.