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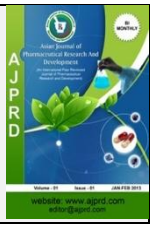

Open Access **Research Article**

Antagonistic Activity of *Agaricuscampestris* mushroom Extracts Against some Human Pathogenic Bacterial Species

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A B S T R A C T

The present study focused on the evaluation of *Agaricuscampestris mushroom extracts* activity against four clinical isolates *Escherichia coli; Pseudomonas aerginosa*; *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Agaricuscampestris mushroom extracts* varied in their antimicrobial activity. The intensity of the antimicrobial effect is dependent upon the solvent , concentration of the mushroom extract and the organism being tested against .Therefore, The hot water and ethanolic extract 96%showed maximum inhibition at high concentrations specially at 500 mg/ml concentration(17, 16, 19, 16) mm and (20,21,19,20) against *E. coli , Pseudomonas aerginosa*; *Staphylococcus aureus* and *Staphylococcus haemolyticus* respectively compared with methanolic extract . The ethyl acetate extract of the powder mushroom of *Agaricuscampestris* was analyzed to 9 chemical compounds belonged to fatty acids and their derivatives and included :-Nonadecane, Pentacosane, Octadecane, Heptadecane, Heneicosane, Hexadecane, 2,6,10,14-tetramethyl, Butanoic acid, 3,7-dimethyl-6-octenyl ester, Phthalic acid mono-2-ethylhexyl ester ,, Octanoic acid, heptadecyl ester.

Keywords:- antimicrobial activity , *A.campestris* , GC_MS

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INTRODUCTION

Some mushrooms are edible, such as the genera of *Agaricus* , *Pleruotus*, *Lentinus*, *Gonoderma* , while other mushrooms are extremely poisonous, such as *Amanita phalloides.* A number of mushroom varieties have global economic importance, through being all year round cultivated delicacies considered as food and medicine..Medicinal mushroom application can be through concentrates or powdered forms on hot water extracts and essences, which are applied as alternative medicine .Both the edible and non-edible wild mushrooms have antibacterial properties. Inhibition of microbial growth by mushroom extracts is due to the presence of bioactive components in the mushrooms[1]. Aqueous, ethanol, methanol of some species of *Agaricus* have been reported to exhibit antibacterial activity against *E. coli*, *Enterobacteraerogenes*, *Pseudomonas aeruginosa* and *Klebsiellapneumoniae*. Most of the species of showed antimicrobial activity against bacteria and yeasts[2,3]. There is urgent need to seek nature friendly alternatives to these synthetic chemicals and mushrooms are good source of these alternatives[4]. Methanol extracts of wild edible mushrooms, namely *Agaricusarvensis*, *Agaricuscampestris*, *Armillariamellea*, *Fomesfomentarius*, *Coprinusmicaceus*, *Coriolusversicolor* and *Lactariusdeliciosus* possess antibacterial and antioxidant properties [5].Extracts of three mushrooms, *Terfeziaboudieri*, *Agaricusbrunnescens* and *Lactariusvellereus* were tested for their antibacterial potency against bacteria. Therefore, the present study focused on the evaluation of antibacterial activities of hot water, methanol and ethanol extracts of *Agaricuscampestris* mushroom using the agar well diffusion method against four clinical bacterial isolates two

negative bacteria *Escherichia coli*, *Pseudomonas aerginosa* ,and two positive bacteria *Staphylococcus aureus* ,*Staphylococcus haemolyticus* in addition to chemical analysis of dried powder fruiting bodies of *A. campestris* using by GC-MS.

METHODS

The References of strains of Edible Mushrooms

Wild mushroom of *A. campestris* was brought from market of Malaysia placed in sterile Polyethylene bags. The dried mushrooms were then ground to powder using an electrical grinder (Singsung - Singapore) to obtain a fine powder and preserved at room temperature until the use

Preparation of Mushroom Extracts

A fine dried powder of *A.campestris* mushroom (10 g) was extracted sequentially by soaking with 150 ml of of hot water, ethanol 96% or methanol 96% and was shaken using an incubator shaker at 150 rpm for 72 h. The extracts were centrifuged at 3000 rpm for 15 min, filtered with Whatman No.1 filter paper, and evaporated and driedusing incubator at 37 ºC . The extracts were kept in the 4◦C refrigerator . stock solutions of each ethanol and methanol mushroom extracts were prepared by dissolving in 10% dimethyl sulfoxide (DMSO) ,while the hot water dissolved by sterilized distilled water , then prepared different concentrations (100%, 200% ,300%, 400% and 500% from The stock solution 1000mg /ml .

The References of Bacteria Used for Antimicrobial Activity Test

The identified of four e pathogenic bacterial isolates were obtained from the laboratories of Biotechnology, College of science, Al-Nahrain University isolated from infected patients,as shown in (Table 1).

Table -1-Bacteria used in current study.

No.	Bacteria	Gram stain	Site of isolation
1	*Escherichia coli*	Gram negative	Urine
2	*Pseudomonas aerginosa*	Gram negative bacteria	Urine
3	*Staphylococcus aureus*	Gram positive	Blood
4	*Staphylococcus haemolyticus*	Gram positive	Urine

Determination of Antibacterial Activity of Crude Extracts

An agar well diffusion method[6] was used for screening of mushroom alcoholic (ethanol 96% and methanol 96%) and aqueous extracts (hot water) of *Agaricuscampestris* against some pathogenic bacteria, nutrient agar medium was used during this examination. nutrient agar medium was autoclaved at 121°C for 15minutes and poured into Petri dishes. Bacteria were grown in nutrient broth for 24 hr. The overnight culture suspensions were adjusted by comparing alongside with 0.5 McFarland turbidity standard tubes. A 10µl of bacterial suspension was spread by sterile swabs on each nutrient agar plates. Seven agar wells of 5 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. Wells in each plate were loaded with 10 µl of prepared extracts (ethanol, methanol and water) of *Agaricuscampestris* two controls well containing positive control the antibiotic and negative control. The plates were incubated at 37ºC for 24hr in the incubator. The zones of inhibition were measured as the diameter of the inhibition around the wells (in mm) including the well diameter using a ruler.

Preparation of an ethyl acetate fraction from *A.campestris* mushroom

The dried powder of *A.campestris* mushroom (10 g) were extracted with 80% aqueous methanol (50mL) and 50% aqueous methanol (50mL) at, the extracts were combined and concentrated on a rotary evaporator under reduced pressure at room temperature until the methanol was removed. The aqueous extract was partitioned with ethyl acetate two times. The combined ethyl acetate fraction was evaporated under reduced pressure at room temperature followed by drying in a freeze-dryer kept at -20 ºC until the use [7].

Analysis of ethyl acetate extracts by GC-MS

GC-mass chromatography analysis was performed to identify the chemical compounds in ethyl acetate extracts of *A.campestris* Identification of chemical compounds was done by injecting 2µl of sample into an RT * 5 column (30 * 0.32 nm) of GC-MS model (Perkin Elmer, Clarus 500, USA);helium(3ml/ min) was used as a carrier gas. The following temperature gradient program was used (75 °C for 2 min followed by an increase from 75to175 °C at a rate of 50 °C per min and finally 7 min at 175 °C).The m/z peaks representing mass to charge ratio characteristics of the chemical compounds fractions were compared to those in the mass spectrum library of the corresponding organic compounds. This experiment was conducted in Science and Technology Ministry.

RESULTS

Antibacterial activity of *Agaricuscampestris* Mushroom Extracts against some Pathogenic Bacteria

The hot water extract of edible mushroom *Agaricuscampestris* was screened against four pathogenic bacteria *S. aureus*, *Staph. Haemolyticus*, *Pseudomonas areuginosa* and *E. coli*. The results hot water extract of *A.campestris* showed growth inhibition of four species bacteria in high concentrations (300, 400, 500) mg/ml as seen in (Table 2).It showed maximum inhibition of 17 mm and 19 mm at 500% concentration of the extract against *E. coli* and *aureus* respectively, compared with other concentration and positive control.

Table 2: Antibacterial activity of hot aqueous extract of A.campestris

Pathogenic bacteria	Treatments Diameter of Inhibition zone (mm) Treatments mg/ml						
	100	200	300	400	500	Con-	Con +
E.Coli	9	11	13	15	17	0.0	23
Pseudomonas areuginosa	11	12	13	14	16	0.0	23
Staph aureus	9	12	14	17	19	0.0	23
Staph.haemolyticus	9	10	11	14	15	0.0	23

The ethanol extract of *Agaricuscampestris* was screened against *S. aureus* and *E. coli*. The ethanol extract of *Agaricuscampestris* showed variation in growth inhibition of four species bacteria in different concentrations and different types of bacteria species as seen in (Table 3) it showed more effectiveness on growth inhibitionin all pathogenic bacteria at concentration 500mg/ml then the rest of concentrations.

Table-3-Antibacterial activity of ethanolic 96% extract of *A.campestris*

Pathogenic bacteria	Diameter of Inhibition zone (mm) Treatments mg/ml						
	100	200	300	400	500	Con-	Con +
E.Coli	8	12	16	19	20	0.0	23
Pseudomonas areuginosa	10	16	16	19	21	0.0	23
Staph aureus	8	10	14	17	19	0.0	23
Staph.haemolyticus	9	13	15	15	20	0.0	23

Table-4-Antibacterial activity of methanolic 96% extract of *A.campestris*

Pathogenic bacteria	Diameter of Inhibition zone (mm) Treatments mg/ml						
	100	200	300	400	500	Con-	Con +
E.Coli	9	9	11	13	14	0.0	23
Pseudomonas areuginosa	8	9	13	14	14	0.0	23
Staph aureus	8	10	12	15	17	0.0	23
Staph.haemolyticus	9	10	12	12	13	0.0	23

Analysis of the Chemical Constituents *A.campestris* By (GC-MS

The GC-MS chromatography of ethyl acetate extract of dried powder mushroom of *Agaricuscampestris* was separated to the nine peaks of the compounds detected was shown in (Table 5). And (Figure 1). Peaks were determined to be Nonadecane, Pentacosane, Octadecane, Heptadecane, Heneicosane, Hexadecane, 2,6,10,14-tetramethyl , Butanoicacid, 3,7-dimethyl-6-octenyl ester , Phthalic acid mono-2-ethylhexyl ester, Octanoic acid, heptadecyl ester.

Table-5: GC -Ms profile of ethyl acetate extract of *A.campestris* mushroom

NO.	R.T min	Compounds	Molecular formula	Molecular weight (g/mole)	Area%
1	24.031	Nonadecane	$C_{19}H_{40}$	268.5	3.81
2	24.545	Pentacosane	$C_{25}H_{52}$	352.7	7.27
3	24.837	Octadecane	$C_{18}H_{38}$	254.5	8.17
4	25.380	Heptadecane	$C_{17}H_{36}$	240.5	17.85
5	25.620	Heneicosane	$C_{21}H_{44}$	296.6	12.91
6	26.031	Hexadecane, 2,6,10,14-tetramethyl	$C_{20}H_{42}$	282.5	41.51
7	27.414	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{15}H_{28}O_2$	240.3	2.30
8	31.061	Phthalic acid mono-2-ethylhexyl ester	$C_{16}H_{22}O_4$	278.3	4.28
9	35.759	Octanoic acid, heptadecyl ester	$C_{25}H_{50}O_2$	382.7	1.90

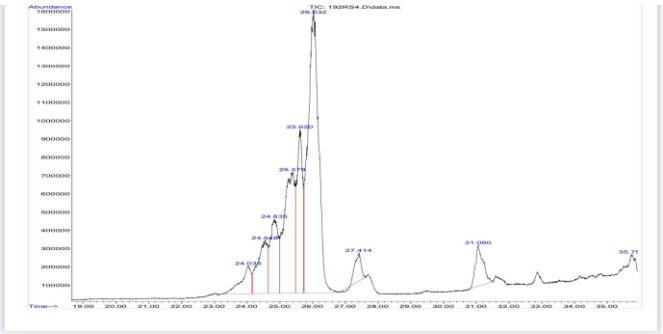

Figure-1: Quantitative and Qualitative Assessment of Active compounds in Ethyl Acetate Etracts of *A.campestris*

DISCUSSION

In antimicrobial activity there were variations in the degree of antimicrobial activities of mushrooms. The broad spectrum activity of mushrooms was also brought to light as the extracts of mushrooms showed inhibitory effects on clinical isolates used for this investigation. This suggests that the bioactive products which are contained in mushrooms are in concentrations which exude varying degrees of antimicrobial activity. Furthermore, the study revealed that the bacterial isolates (*Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Finding is in accordance with the findings of[8,2].It is interesting to note from the results of this study that clinical isolates both Gram positive and Gram negative bacteria were sensitive to the extracts. But the gram positive bacteria showed more sensitivity than gram negative bacteria. This is in collaboration with the findings of[9].Similar studies have evidenced that *genus Agaricus in general has* a broad spectrum of antimicrobial activity. By using solvents with different polarities. Risan and others[8].found that hot water extracts of *Agaricusbisporus* had a stronger inhibitory activity on both Gram-positive and Gram-negative bacteria but with varying degrees of intensity.

The results of the current study are in agreement with the work of theearlier workerswho have also reported strong antibacterial activity of mushroom extract against gram negative bacteria and gram-positive bacteria[10,11,3]. Also another study [12]., also reported the antibacterial potential of ethanolic extract of some species of *Agaricus* .

While in analysis profile of ethyl acetate extract of mushroom the results were mostly considered from hydrocarbon lipid molecules .from this table saturated fatty acids and its derivatives Hexadecane, 2,6,10,14-tetramethyl is the predominant compound in the structures of ethyl acetate extract of *Agaricuscampestris* which it occupied area % about 41.51% compared with other compounds many studies confirmed on the role of fatty acids and their

dervitives as antibacterial agents that destabilize bacterial cell membranes, causing a wide range of direct and indirect inhibitory effects. [13,14,15].

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