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In Vitro Antibacterial Activity of *Hypsizygus ulmarius* (Bull.) Fruiting Bodies

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ABSTRACT

Mushrooms have medicinal value in enhancing immune system and also as antibacterial and anticancer. The present study was aimed to investigate antibacterial and anticancer activity of methanolic extract, petroleum ether and ethyl acetate fractions of *Hypsizygus ulmarius (Bull.)* fruiting bodies. Antibacterial activity was tested against different Gram- negative bacteria using agar well diffusion method. Methanolic extract showed the highest activity against *P. aeruginosa, P. mirabilis, K. pneumoniae, E. faecalis* (ATCC 29212) and *K. pneumoniae* (ATCC 700603). Petroleum ether fraction showed activity on *P. aeruginosa, P. mirabilis, K. pneumoniae and E. coli* (ATCC25922), while ethyl acetate fraction has activity against *P. aeruginosa and K. pneumoniae.* Methanolic extract, *petroleum* ether and ethyl acetate fractions showed no activity against human cervical cell line and epithelial human breast cancer MDA-MB-23.

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INTRODUCTION

Mushrooms have been used by ancient people as a source of foods and medical preparations to treat different types of infections (1-4). Now mushrooms have more attention as they contain medical compounds and nutritional food (5-7). Many of modern scientific research has documented that mushrooms contain unlimited source of bioactive compounds which can be used in drug discovery and development. Many pharmacological agents extracted from mushrooms have been reported to possess activity as anticancer, antimicrobial, anti-inflammatory, immunomodulators, hypocholesterolemia, antiviral, antioxidant, antihyperglycemic and antihypertensive (8-15).

Elm oyster mushroom, *Hypsizygus ulmarius* (*BULL*.), is a high yielding edible mushroom. Medicinal properties of this mushroom have been reported (16). The aim of the present study is to evaluate antibacterial and anticancer activity of *Hypsizygus ulmarius* (*BULL*.) fruiting bodies.

MATERIALS AND METHODS

Mushroom material

Dried *Hypsizygus ulmarius* fruiting bodies were collected from "S'' Mushroom Agritech, Hyderabad, Telangana state, India. Mushroom had been grown at 25^0 and dried by solar method.

Mushroom extraction

Dried fruiting bodies of *H. ulmarius* (650 g) were powdered and extracted with methanol (5 L) using a Soxhlet apparatus (14 cycles). The solvent was completely evaporated using rotary evaporator, yielding an extract (133 g) which is viscous in nature and brown in color (17).

Fractionation of methanolic extract

Methanolic extract (only 90 g), was fractionated using petroleum ether and ethyl acetate, respectively. Both solvent extracts were concentrated using rotary evaporator.

Bacterial isolates

Clinical isolates along with standard bacterial strains of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603) and *Enterococcus* faecalis (ATCC 29212) were collected from Microbiology department, Medical college, Aurangabad, India.

Antibacterial activity of methanolic extract and its fractionation

Bacterial strains were cultured on nutrient agar at 37^0 for 24 h. Then, bacterial suspension was prepared and adjusted to 0.5 McFarland turbidity standard. Agar well diffusion method is used to evaluate antibacterial activity of mushroom extracts (18). Methanolic extract and its fractions were dissolved in 1 % dimethyl sulfoxide (DMSO) to final concentrations of (100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml). Nutrient agar plates were prepared, allowed to solidify and 100 µl of bacterial suspensions were seeded on the surfaces of these plates using cotton swabs. Wells of 8 mm in diameter were bored on the agar using a sterile cork borer. 100 µl of methanolic extract and its fractions were than introduced in to the wells and appropriately labeled. Streptomycin was used as a stander antibiotic (Positive control) and 1% DMSO as a negative control. The plates than incubated at 37^0 for 18-24 h. The diameter of the inhibition zone was measured in millimeters. Studies were performed in triplicate.

Minimum inhibitory concentration (MIC)

To determine MIC, classical double dilution method was used (19). A series of different concentrations for methanolic extract and its fractions were prepared (ranging from 100 mg/ml to 3.125 mg/ml).

Minimum bactericidal concentration (MBC)

To detect MBC, a loopful of nutrient broth tubes which showed no growth were inoculated on to fresh sterile nutrient agar plates. Also, equal volume of fresh sterile nutrient broth was added in to the test tube cultures and inoculated further for 18-24 h at 37^0.

Cancer cell lines

Human cervical cell line (Hela) and epithelial human breast cancer MDA-MB-231 were obtained from cancer research unit, Basavatarakam Indo-American cancer hospital and research institute, Hyderabad, India.

Anticancer assay

To evaluate anticancer activity of methanolic extract, petroleum ether and ethyl acetate fractions on Hela and MDA-MB-231 cancer cell lines, MTT colorimetric assay was applied (20). Briefly, cancer cell lines were seeded in 96-well plates at a density of 5×10^3cells per well and then incubated at 37^0 in 5% CO_2 to allow cell attachment. After 24 hours, the medium was discarded and various concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml and 600 µg/ml) of methanolic extract, petroleum ether and ethyl acetate fractions were added and incubated for 48 hours in 5% CO_2. After incubation, the medium was removed and fresh medium with 100 µL MTT (5 mg/mL) was added to each well and the plate was further incubated for four hours. After that, medium was removed and 100 µL of DMSO was added to each well. Absorbance was read in a spectrophotometer at 570 nm. Results were expressed as the percentage of cell viable compared to control Doxorubicin (20 µg/ml and 200 µg/ml). Cell viability was calculated by the formula:

Cell viability (%) = Test OD /control OD ×100.

Where OD is the optical density.

Statistical Analysis

Experimental data are expressed as means ± stander error. Statistical analyses were performed by one-way ANOVA. This analysis was done using SPSS ver. 20.0 software**.**

RESULTS AND DISCUSSION

Yield of the Methanolic extract and its fractions

The yield and characteristics of methanolic extract, petroleum ether and ethyl acetate fractions are presented in Table 1.

Table No. 1: Characteristics of methanolic extract and its fractions.

Mushroom extract	Weight of extract (g)	Consistency and appearance
Methanol extract	133	Dark brown, semisolid and viscous
Petroleum ether fraction	8	Dark brown, semisolid and sticky
Ethyl acetate fraction	4	Dark brown and waxy

In Shivashankar M and Premkumari B (21) findings, methanolic extract of *H. ulmarius* fruiting bodies was brown, semisolid and sticky which is compatible to our result.

Antibacterial activity

This is the first study conducted to evaluate antibacterial activity of *H. ulmarius* fruiting bodies against Gram negative bacteria. Antibacterial activities of methanolic extract and its fractions at various concentrations are presented in Table 2, 3 and 4 and Figure 1.

Table No. 2: Antibacterial activity of methanol extract

Bacterial Species	Methanolic extract (Inhibition zone (mm))				Streptomycin
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml
P. aeruginosa	11.0 ± 0.58	12.67 ± 0.33	13.67± 0.33	16.67 ± 0.33	23.67 ± 0.88
P. mirabilis	17.67±0.88	20.67 ± 0.67	22.33 ± 0.67	24.33 ± 0.67	30.33± 0.33
K. pneumoniae	17.0 ± 0.58	21.33 ± 0.33	21.67 ± 0.33	21.67 ± 0.33	28.67± 0.33
E. faecalis **(ATCC 29212)**	21.0 ± 0.58	22.0 ± 0.58	23.67 ± 0.88	25.33 ± 0.33	32.67 ± 1.20
K. pneumoniae **(ATCC700603)**	13.67± 0.88	18.67 ± 0.33	20.33 ± 0.33	22.0 ± 1.0	33.67±0.88

All values are expressed as Mean ± SEM (n = 3).

Table No. 3: Antibacterial activity of petroleum ether fraction.

Bacterial Species	Petroleum ether fraction (Inhibition zone (mm))				Streptomycin
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml
P. aeruginosa	10.67 ± 0.33	12.0± 0.58	14.67± 0.33	18.67 ± 0.33	20.33 ± 0.33
P. mirabilis	18.33 ± 0.33	18.33 ± 0.33	21.0 ± 0.58	22.67 ± 0.33	29.0± 0.58
K. pneumoniae	0.0 ± 0.0	11.67 ± 0.33	16.0 ± 0.58	18.33 ± 0.33	23.33± 0.33
E. coli **(ATCC25922)**	13.67 ± 0.88	16.33 ± 0.33	18.67 ± 0.33	18.67 ± 0.33	27.67 ± 0.33

All values are expressed as Mean ± SEM (n = 3).

Table No. 4: Antibacterial activity of ethyl acetate fraction.

Bacterial Species	Ethyl acetate fraction (Inhibition zone (mm))				Streptomycin
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml
P. aeruginosa	18.0 ± 0.58	20.33± 0.33	20.33± 0.33	20.33± 0.33	25.33 ± 0.33
K. pneumoniae	19.67 ± 0.33	20.0 ± 0.58	20.33 ± 0.88	21.67 ± 0.33	24.67± 0.33

All values are expressed as Mean ± SEM (n = 3).

In screening step, methanolic extract was more active than its fractions. It showed activity on *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *E.* faecalis and *K. pneumoniae* ATCC 700603 (Table 2, Fig 1: A, B, C, D, E). The greatest inhibition zone (25.33 mm) was obtained from the methanol extract against *E.* faecalis at concentration of 400 mg/ml. At concentrations, 300 mg/ml and 400 mg/ml extract showed no differences in activity against *K. pneumoniae* in which inhibition zone was 21.67 mm.

Petroleum ether fraction activity are shown in Table 3. It showed activity against *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae* and *E. coli* (Fig 1: F, G, H, I). The highest activity was against *P. mirabilis* at concentration 400 mg/ml (22.67 mm inhibition zone). There was no difference on its activity on *P. mirabilis* at concentration 100 mg/ml and 200 mg/ml (18.33 mm) and also on *E. coli* at concentrations 300 mg/ml and 400 mg/ml (18.67 mm). The ethyl acetate fraction showed inhibition activity only on *P. aeruginosa* and *K. pneumoniae* (Table 4, Fig 1: J, K). The highest inhibition zone was 21.67 mm at concentration 400 mg/ml for *K. pneumoniae*. At concentrations 200 mg/ml, 300 mg/ml and 400 mg/ml ethyl acetate fraction showed no difference in activity against *P. aeruginosa*. Antibacterial activity increases gradually as dose is increased. The commercial antibiotic was more effective in their antibacterial activity.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) values which ranged from 3.125 to 100 mg/ml and minimum bactericidal concentration (MBC) are represented in Table 5. The Lowest MIC was noticed in methanolic extract against *P. mirabilis*, *K. pneumoniae*, petroleum ether fraction against *P. mirabilis* and in ethyl acetate fraction against *K. pneumoniae* (3.125mg/ml). For the rest of bacteria, it's varying from 6.25mg/ml to 12.5mg/ml.

Table No. 5: MIC and MBC of methanolic, petroleum ether and ethyl acetate fractions.

Bacterial species	Methanolic extract		Petroleum ether fraction		Ethyl acetate fraction	
	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml
P. aeruginosa	6.25	12.5	6.25	12.5	6.25	12.5
P. mirabilis	3.125	6.25	3.125	6.25	-	-
K. pneumoniae	3.125	6.25	12.5	12.5	3.125	6.25
E. faecalis	6.25	12.5	-	-	-	-
***K. pneumoniae* (ATCC 700603)**	6.25	6.25	-	-	-	-
E. coli	-	-	6.25	12.5	-	-

Note: (-) extracts have no activity.

Anticancer activity

Methanolic extract, petroleum ether and ethyl acetate fractions showed no anticancer activity against Hella and MDA-MB-231 cancer cell lines.

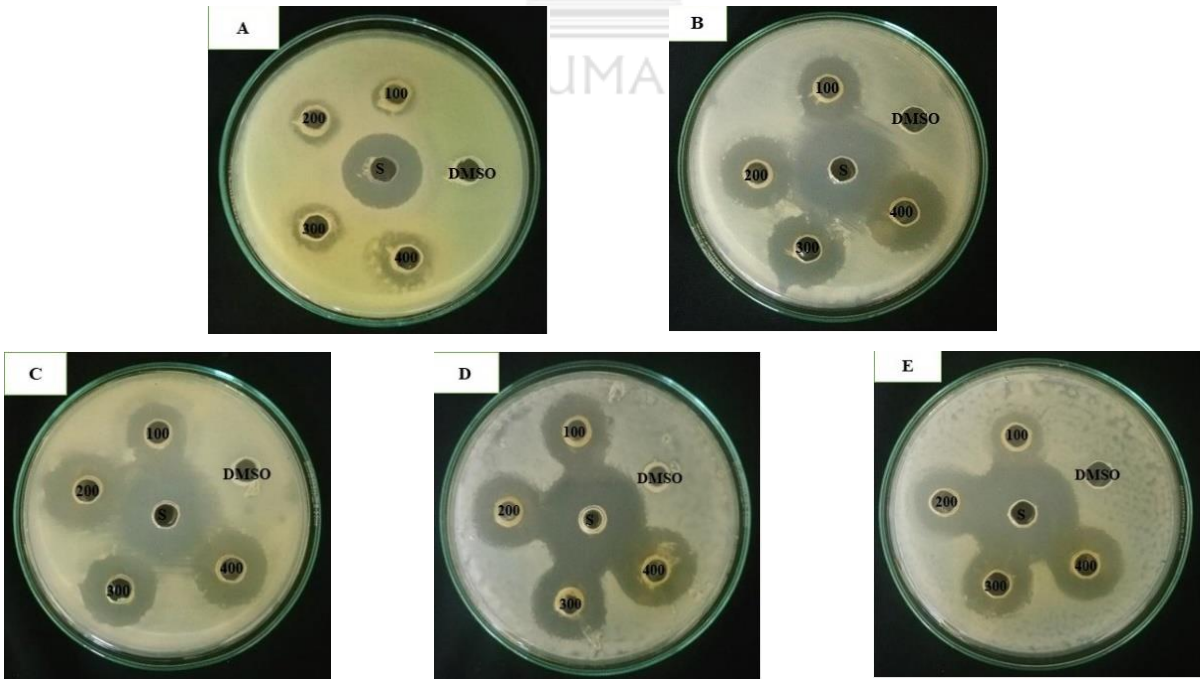

Figure No. 1: Antibacterial activity of methanol extract on (A) *P. aeruginosa*, (B) *P. mirabilis*, (C) *K. pneumoniae*, (D) *E. faecalis* and (E) *K. pneumoniae* (ATCC 700603).

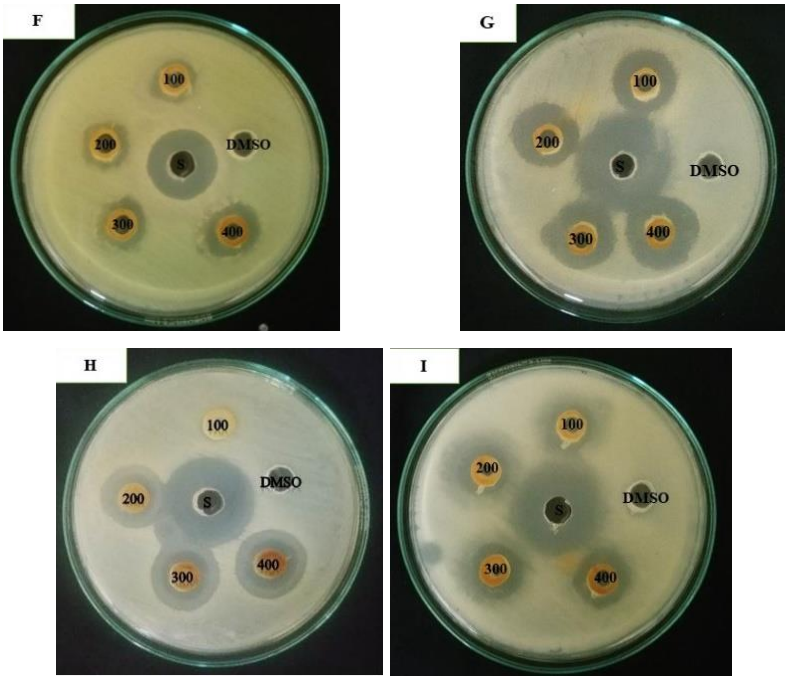

Figure No. 2: Antibacterial activity of petroleum ether activity on (F) *P. aeruginosa*, (G) *P. mirabilis*, (H) *K. pneumoniae* and (I) *E. coli*.

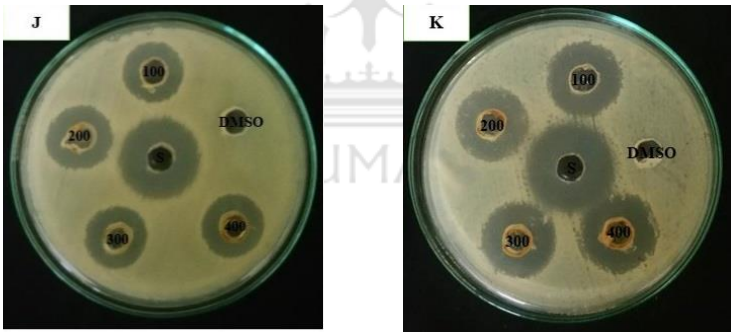

Figure No. 3: Antibacterial activity of ethyl acetate activity on (J) *P. aeruginosa* and (K) *K. pneumoniae*.

CONCLUSION

This is the first study that conducted to evaluate antibacterial activity of *H. ulmarius* fruiting bodies. We found that methanolic extract have a good activity against selected Gram-negative bacteria in compare to its fractions. Methanolic extract and its fractions have no anticancer activity on the selected cancer cell lines. Further work needs to be investigated by using higher dose of extract and its fractions against used cancer cell lines and also on another types of cancer cell lines.

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