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EVALUATION OF ANTIBACTERIAL ACTIVITY OF MUSHROOM *PLEUROTUS OSTREATUS* EXTRACT AGAINST EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING MICROORGANISMS

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ABSTRACT

Backrround: Extensive use of antibiotics, followed by non compliance of the patient resulted in the development of more than one drug resistant bacterium. They are responsible for rapid outbreaks and are primary cause for nosocomial infection. Extended spectrum beta lactamase **(**ESBL) positive strains are resistant to 3rd and 4th generation cephalosporins and monobactams. **Aim:** To study the antibacterial efficacy of the oyster mushroom *Pleurotus ostreatus* extract against six ESBL positive isolates. The detection of different phytochemicals constituent of the crude extract was also taken into consideration. **Methods:** The antibacterial efficacy of the oyster mushroom *Pleurotus ostreatus* was evaluated against six ESBL positive isolates via agar disc diffusion assay, minimum inhibitory concentration assay (MIC), minimum bactericidal concentration assay (MBC). The phytochemical composition of the crude extract was assayed by UV-Vis spectrophotometry. **Results:** Six isolates of ESBL producing bacteria were cultured from various clinical samples of patient after taking informed consent. The zone of inhibition of the extract of mushroom lies within 11mm – 12mm. MIC study have revealed the potent antibacterial efficacy of the mushroom extract against the ESBL positive isolates. The MBC values of the mushroom extract lies in the range 10 – 21mg/mL against the ESBL positive isolates. The mushroom extract was scanned in the range 200 – 800nm by UV-Vis spectrophotometry and it showed the presence of

probable phytochemicals based on peak analysis. **Conclusion:** Oyster mushroom *P. ostreatus* showed potent antibacterial efficacy against ESBL producing bacteria. Therefore oyster mushrooms can be taken as dietary supplements along with our daily nutrition.

KEYWORDS: Mushroom, ESBL producing microorganisms, antibacterial activity, UV-Vis spectrophotometry.

INTRODUCTION

Extended spectrum beta lactamase (ESBL) producing strains were first detected in mid 1980s in Western Europe. They are able to hydrolyze 3rd and 4th generation cephalosporins and monobactams but these strains get inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.[1,2] An initial outbreak by these organisms was followed by endemecity in some hospitals. ESBL are group of enzymes encoded by plasmids which are common among Enterobacteriaceae.[3] Most of the ESBL enzymes are mutants of temoneira (TEM) and sulfhydryl variable enzymes (SHV), the cefotaximase (CTX-M) type lactamase, originated from β-lactamases of genus *Kluyvera* hydrolyzes cefotaxime and ceftriaxone but weakly active against ceftazidime.[4,5] Carbapenems represents a great choice for the treatment of infections caused by beta-lactam resistant bacteria.[6] The exhaustive use of carbapenems, poor sanitation and huge population facilitated the emergence of carbapenem resistant bacteria.[7] These isolates when present in ICU and in hospital environment poses a serious threat to treatment and infection control management systems.[8] Therefore there is an utmost need to discover new drugs against these microorganisms from any possible sources. Many antibacterial and antifungal compounds are naturally present in mushrooms to help them survive in the environment. It is known to everyone that usually some compounds from microscopic fungi are marketed as antibiotics till date.[9] There is recent upsurge in interest among various researchers regarding the antimicrobial action of active compounds present in mushrooms showing action against multidrug resistant bacteria. It was observed that new sesquiterpenoid hydroquinones isolated from *Ganoderma pfeifferi*, which was named ganomycin, can inhibit the growth of methicillin – resistant *Staphylococcus aureus* and other bacteria.[10] The cap of the mushroom Pleurotus sp. is more like an oyster in shape and therefore it is known as oyster mushroom. It usually grows in clumps, it may be 3-6 inches wide, upper surface is white or ivory coloured and is smooth in texture. It is fleshy, soft and spongy. An unwanted feature of this particular mushroom is that it decays rapidly. They generally grow on dead woods or logs specifically in autumn.[11] Therefore our objective was

to analyse the antibacterial potency against ESBL producing microorganism. Moreover we have also evaluated the probable phytochemicals present in the crude extract of mushroom *Pleurotus ostreatus*.

MATERIALS AND METHOD

- **Reagents Used:** Standard antibiotic discs were purchased from Hi-Media Laboratory Ltd. (Mumbai, India).

- **Mushroom Sample Collection:** The fresh edible oyster mushroom *Pleurotus ostreatus* was identified and collected from cultivation farm, maintained by Ramkrishna Mission, Narendrapur, Kolkata.

- **Microorganism used for the study:** A total of six ESBL positive microorganisms were isolated and cultured from various clinical samples and maintained by preparing cryovial for long term assay procedure. These microorganisms were identified by the standard microbiological techniques and also by the automated panels of system Vitek® 2 (Biomeiurieux, USA) .Clinical histories of the patient samples were recorded for future analysis. *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883) were used in our study as reference culture.

- **Detection of ESBL isolates by phenotypic detection method:** Phenotypic detections were done as per the recommendations of CLSI guidelines. Kirby Bauer disc diffusion assay was performed. The combination of ceftazdime and clavulanic acid (30/10 µg discs) along with ceftazidime alone were used for ESBL detection. The assay was performed on Mueller Hinton agar and the plates were incubated for next 16 -18 hours. Any distortion or increase (≥ 5mm) in the zone towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production.[12]

- **Preparation of extract:** Fresh edible mushroom samples were collected and shade dried under the sun for 72 hrs. The dried mushroom sample was grinded using electrical grinder. 10 g of powdered sample was extracted with 100 mL of 60% (v/v) aqueous ethanol, for 72 hours following the solvent extraction method. Then the extract was passed through Whatman filter paper No. 1 and the clear filtrate was again filtered with 0.22 micron syringe filter and the content was lyophilized and stored at 4°C.[13]

• **Analysis of Moisture Content and Yield Analysis of the crude bioactive content from mushrooms:** The fresh edible mushroom sample was weighed immediately after the collection and another weight (in gms) were recorded after shade drying under the sun. The percentage yield was obtained using the formula:

W_2-W_1 / W_0 X 100

Here W_2 is the weight of the extract and the container, W_1 the weight of the container alone and W_0 the weight of the initial dried sample.[14]

• **Evaluation of Antibacterial activity of extracts against the microorganisms**

Antibacterial activity was assayed using Agar disc diffusion assay, Minimum Inhibitory Concentration assay (MIC) and Minimum Bactericidal Concentration Assay (MBC) following standard CLSI guidelines.[15]

• **Characterization of hydroethanolic extracts using UV-Vis Spectroscopy**

The ethanol extracts of all mushrooms were scanned within the wavelength between 200 – 800 nm using UV- Vis spectrophotometer (Perkin elmer). Probable phenolics and flavonoids present in the extracts were evaluated after analysis of the peak.[16]

RESULTS

Fig. 1: *Pleurotus ostreatus* (Oyester mushroom, edible).

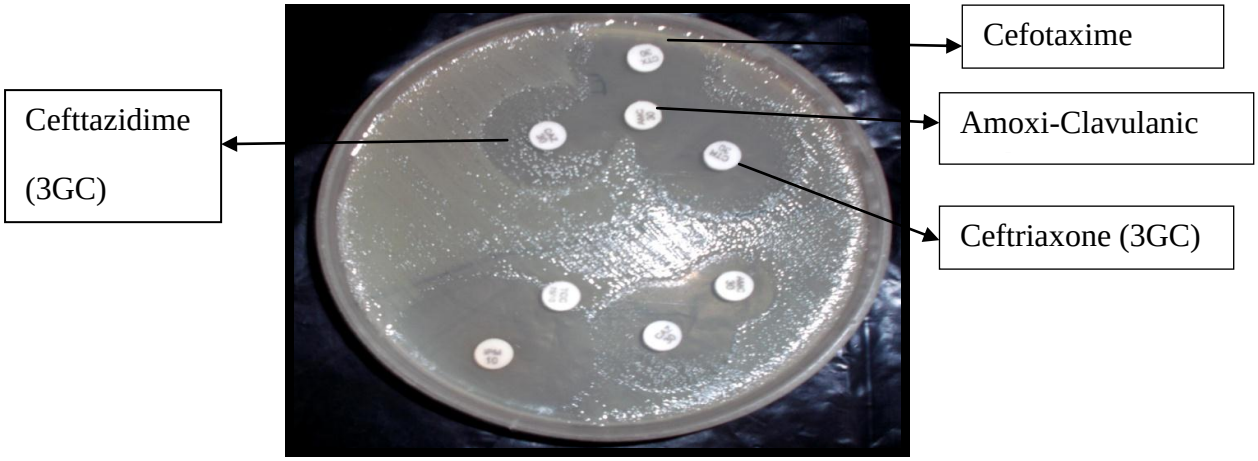


Fig. 2: Phenotypic detection of ESBL positive *Klebsiella pneumonia* by double disk synergy method.

Fig. 3: (Left) Hydroalcolic extract prepared from the powdered mushroom sample using solvent extraction method using 60% (v/v) aqueous ethanol.

Fig 4: (Right) Crude content of the extract was dissolved at 10% DMSO.

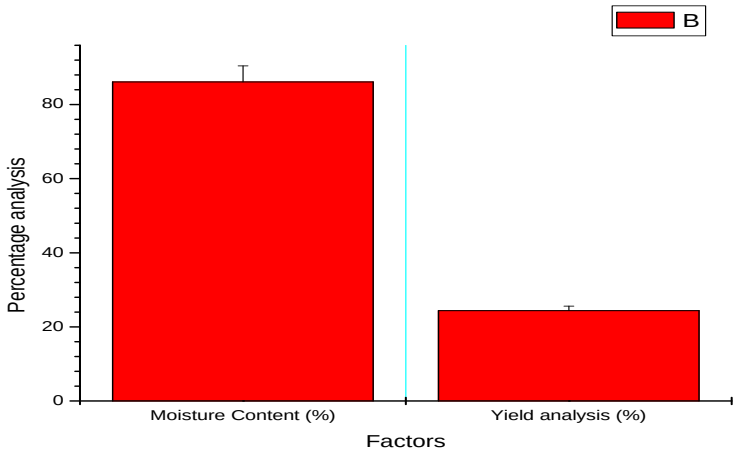


Fig. 5: The figure represents the mean ± SD moisture content (%) and yield content (%) of the edible mushroom *Pleurotus ostreatus.*

Table. 1: The mean of zone of inhibition (mm) obtained against the multidrug resistant isolates.

Sl. No.	Microorganism	Zone of inhibition (in mm)
1	ESBL isolate 1	12
2	ESBL isolate 2	11
3	ESBL isolate 3	12
4	ESBL isolate 3	12
5	ESBL isolate 4	12
6	ESBL isolate 5	11
7	ESBL isolate 6	11
8	*E. coli* (ATCC 25922)	15
9	*K. pneumoniae* (ATCC 13883)	12

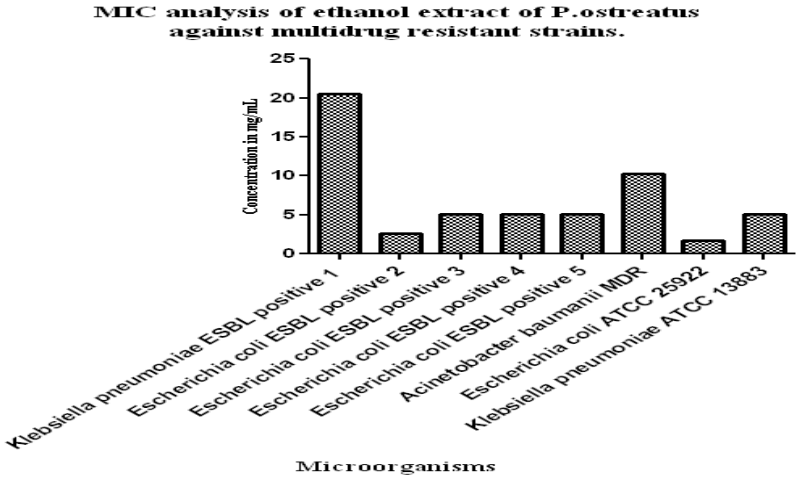


Fig. 6: The bar diagram represents the mean MIC (mg/mL) of the ethanol extracts of mushrooms *P. ostreatus* against the selected multidrug resistant strains respectively.

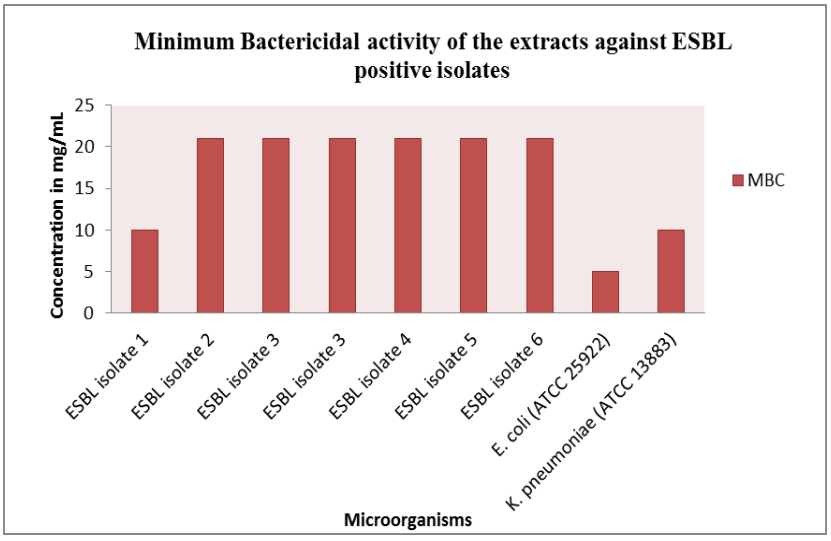


Fig. 7: The bar diagram represents the mean MBC (mg/mL) of the ethanol extracts of mushrooms *P. ostreatus* against the selected multidrug resistant strains respectively.

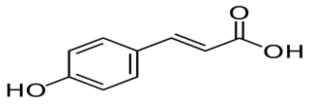
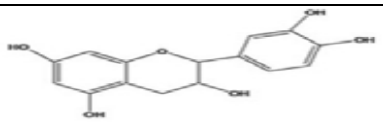
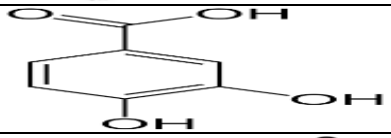
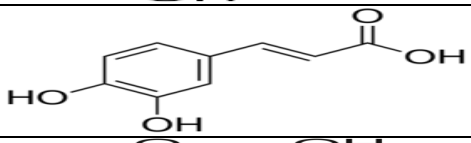
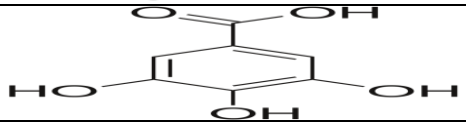
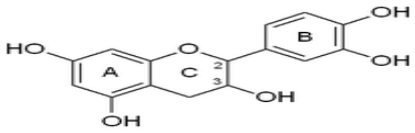
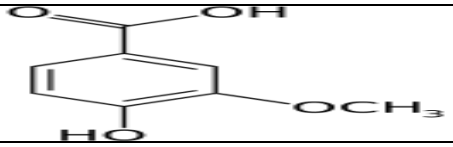
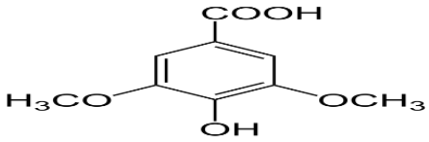
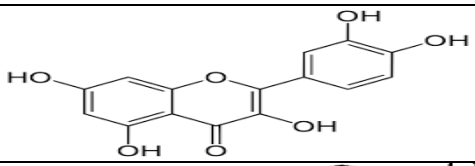
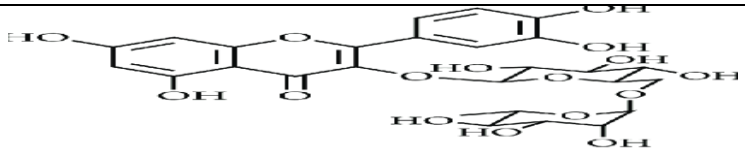
Table. 2: Clinical histories of patients are given below in table format.

Sl. No.	Microorganisms	Age of the patient (in years)	Sex of the patient	Patient History
1.	ESBL isolate 1	68	Male	Diabetic, Pus cells observed in urine culture.
2.	ESBL isolate 2	66	Female	Diabetic, 1-2 Pus cells observed in routine urine culture.
3.	ESBL isolate 3	56	Female	Diabetic Patient with Urinary tract infection. Plenty of pus cells observed.
4.	ESBL isolate 3	40	Female	Diabetic Patient with Urinary tract infection. 3-5 pus cells / Epithelial cells 1-3 observed.
5.	ESBL isolate 4	22	Male	Pus cells, Epithelial cells (1-2) observed in Urine culture..
6.	ESBL isolate 5	76	Female	Haemoglobin 10; Creatinine 1.3; Albumin +ve, Pus cells observed in urine culture.

Table. 3: Antibiotic Resistance profiles along with the clinical source of microorganisms are given below in the table format.

Sl. No.	Microrganisms cultured	Clinical Samples	Detailed Antibiotic Resistance profile
1	*Escherichia coli* ESBL Positive 1	Blood	Augmentin, Aztreonam, Ceftazidime, Ciprofloxacin, Ceftriaxone, Cefuroxime, Cefotaxime, Co-Trimoxazole, Cefepime, Nalidixic acid.
2	*Escherichia coli* ESBL Positive 2	Blood	Augmentin,Aztreonam, Cefoperazone, Ceftazidime, Ceftriaxone, Cefuroxime, Cefotaxime, Ciprofloxacin, Co-Trimoxazole, Levofloxacin, Nalidixic acid, Nitrofurantoin, Netilmicin, Sparfloxacin, Cefepime.
3	*Escherichia coli* ESBL Positive 3	Urine	Augmentin, Azithromycin, Furazolidone, Ceftriaxone, Cefuroxime, Cefotaxime, Ciprofloxacin, Gentamicin, Nalidixic acid, Ofloxacin, Sparfloxacin, Cefepime.
4	*Escherichia coli* ESBL Positive 4	Urine	Augmentin, Azithromycin, Cefuroxime, Cefotaxime, Ciprofloxacin, Co-Trimoxazole, Nalidixic acid, Ofloxacin, , Sparfloxacin, Cefepime
5	*Klebsiella pneumonia* ESBL Positive 4	Urine	Augmentin, Aztreonam, Ceftazidime, Ciprofloxacin, Co-Trimoxazole, Nalidixic acid, Ofloxacin, Cefepime, Sparfloxacin.
6	*Acinetobacter baumannii*	Blood	Ampicillin, Amikacin, Amoxyclav, Cefotaxime,Ceftazidime,Co-Trimoxazole,Ticarcillin-clav, Cefepime, Ciprofloxacin

Table. 4: Probable phenolics and flavonoids present in the extracts were.

Sl. No.	Putative ID of compounds present in the crude mushroom extract	Structure of Probable phenolics
1	p -coumaric acid	
2	epicatechin	
3.	protocatechuic acid	
4.	caffeic acid	
5.	gallic acid	
6.	catechin	
7.	vanillic acid	
8.	syringic acid	
9.	quercetin	
10.	rutin	

DISCUSSION

Large number of outbreak of infections was observed on every continent of globe due to the increasing number of the ESBL producing superbugs. Inactive antibiotics against these microorganisms used in the hospitals resulted in increased patient mortality. Therefore there is an urgent need to control the outbreak of infection in a specialized unit of hospital due to the rapid generation of these microorganisms.[17,18,19]

Existing literature revealed that wild mushrooms were used as an alternative approach against clinical isolates of pathogenic microorganisms. In our study we have analysed the antibacterial potency against six ESBL positive strains along with reference strain. Patients histories were also recorded after taking consent from each of them and the detailed drug resistance profile of the six ESBL positive isolates have also been evaluated (Table 2,3). The literature survey data showed that majority of the extracts of mushrooms namely *Agaricus arvensis*, *A. bisporus*, *Cantharellus cibarius*, *Fistulina hepatica*, *Lactarius deliciosus*, *Lactarius salmonicolor*, *Lepista nuda*, *Leucopaxillus giganteus*, *Mycena rosea*, *Ramaria botrytis*, *Russula delica*, *Sarcodon imbricatus*, *Tricholoma portentosum* did not have any antibacterial activity against Gram negative bacilli such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumanii* used in the study. Extracts of *R. delica* and *F. hepatica* exhibited antibacterial activity against *E. coli*, *M. morganni*, *P. multocida* at concentration of 20mg/mL.[20] Another study showed that methanolic extracts of A. bisporus and C. cibarius have high antibacterial activity against E. coli by disc diffusion method[21]. In our study hydroethanolic (60% v/v) extract of mushrooms P. ostreatus (Fig: 1) showed potent antibacterial activity based on zone of inhibition values (in mm), MIC and MBC values (in mg/mL) (Table 1; Fig: 6-7) against the ESBL positive superbugs (Fig 2). The results of this study also illustrated potential antibacterial activity of the edible mushrooms against the multidrug resistant strains. The moisture content of the oyster mushrooms was found to be 84 % (Fig: 5). The solvent extraction process was carried out at ambient temperature of 25º C (Fig: 3 and Fig. 4). Here in this study we report the extraction yield (%) from mushroom *P. ostreatus*, 24.4% respectively (Fig: 5). The yield content was comparatively higher with respect to previous study conducted (Fig: 5). The UV-Vis spectroscopy study was done to analyze the detailed constituents of the crude extracts. Many important active constituents were found such as protocatechuic acid, quarcetin, gallic acid, coumaric acid, sinapic acid, apigenin, rutin (Table 4).

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CONFLICT OF INTEREST: The authors declare none.

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