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Original Research Article

Antimicrobial Screening of Mushroom Metabolites against Antibiotic Resistant Pathogens

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A B S T R A C T

The present study focused on antimicrobial screening of bioactive metabolites of mushrooms and *Pleurotus sajor, Pleurotus sapidus, Pleurotus djamor, Pleurotus florida* and *Agaricus bisporus* against antibiotic resistant bacterial pathogens. The pathogenic microorganisms were isolated from different clinical samples and were biochemically characterized. The pathogens' antimicrobial susceptibility was tested, by Kirby-Bauer disc diffusion test and CLSI guidelines. The results have revealed a resistance profile. The cultures have exhibited resistance ranging from two to seven antibiotics used in study. Presumptive *Klebsiella sp*. has shown no zone to six antibiotics and a 6mm zone to nimuslide, which however leads to it being resistant to all. Presumptive *Micrococcus sp*., *Pseudomonas sp*., and *Enterobacter sp*., have exhibited resistance to five antibiotics. *Staphylococcus aureus* has shown resistance to four antibiotics. Thus, the microorganisms are gaining resistance to antibiotics whatever be their mode of action. The extracts of mushroom were prepared in different organic solvents and water and their antimicrobial efficacy was put to test against these antibiotic resistant bacteria. The fruiting body extracts of *Pleurotus sajor* and mycelia extracts of *Pleurotus sapidus* have promising antimicrobial activity against antibiotic resistant *Staphylococcus aureus.* The water extracts have given good results in some mushrooms than organic solvent extracts. On the same account *Escherichia coli* has shown zone of inhibition only in acetone extract of *Pleurotus djamor*. However the highest zone of inhibition observed in mushroom extracts is higher than that obtained by antibiotics. Thus, the study has exhibited that the mushroom extracts are effective against both gram-positive and gram-negative microorganisms. Also, different extracts show varying efficacy against pathogenic microorganisms.

Keywords

Antibiotic resistance, mushroom, bioactive metabolites, antibiotic activity

Introduction

Throughout human health history, there has been a continual battle between human and the multitude of microorganisms that cause infection and disease. Beginning around the middle of the 20th century, major advances in antibacterial drug development and other means of infection control helped turn the tide in favour of humans As antimicrobial usage increased, so did the level and complexity of the resistance mechanisms exhibited by bacterial pathogens.

Antimicrobial resistance has emerged as a major health concern with no section of

society or country being immune to the phenomenon. Antimicrobial resistance is a natural survival mode developed by bacteria over a period of time, ensuring its survival amidst various metabolic pathways targeted by antibiotic. However, human factors like overuse, misuse, suboptimal drug use, exposure of patient to poor quality medicines, expansion of counterfeit medicines etc have exacerbated the situation. This has jeopardized the treatment of common infections both at community level and healthcare establishments resulting in complications, disabilities and increasing rate of mortality (Shrivastava *et al.,* 2015, WHO)

World Health Organisation and Centers for Disease Control and Prevention have enlisted "priority list of pathogens" which impose urgent, serious, concerning threats and drug development has to be kept in priority list against them. Those imposing serious threats include members of Enterobacteriaceae multidrug resistant *Pseudomonas aeruginosa*, Methicillicin resistant *Staphylococcus aureus* among others. The above mentioned bacteria cause severe and often deadly infections as bloodstream infections and pneumonia. Therefore, the research of new antimicrobial substances/ alternatives effective against antibiotic resistant pathogenic microorganisms is crucial (https://www.cdc.gov/drugresistance/biggest-threats.html, https://www.who.int/bulletin/volumes/98/3/20-251751/en/)

Mushrooms are saprophyte fungi belonging to the class of Basidomycetes. "Mushroom" is not a taxonomic category. Chang *et al.,* defined the mushrooms as "a macro fungus with distinctive fruiting bodies that could be hypogenous or epigeous, large enough to be seen by naked eyes and to be picked by hands" (Chang and Miles, 1992). In order to endure in their natural environment, mushroom needs antibacterial and antifungal compounds (Lindequist 2005). Mushrooms have been reported to exhibit biological activities that may promote human health benefits. These include antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory properties. Various cellular components and secondary metabolites have been isolated from fruiting bodies, culture mycelium and culture broth of mushrooms.

These include polysaccharides, proteins and their complexes, phenolic compounds, polyketides, triterpenoids, steroids, alkaloids, nucleotides etc. (Valverde *et al.,* 2015).

The phenolic and tannin constituents of mushrooms have found to elicit antibacterial activity with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and micriobial adhensins (Cowan, 1999, Cristiane *et al.,* 2016).

Considering the great interest for mushrooms as source of bioactive metabolites the aim of present study was to investigate the antimicrobial activities of fruiting body extract of *Pleurotus sajor, Pleurotus djamor, Pleurotus florida* and *Agaricus bisporus* and mycelium culture extracts of *Pleurotus sapidus* against antibiotic resistant clinical isolates.

Material and Methods

Collection of mushroom

The fruiting body of *Pleurotus sajor, Pleurotus florida, Pleurotus djamor, Agaricus bisporus* and spawn of *Pleurotu*s *sapidus* were collected from Mushroom Laboratory of Sardar Vallabh Bhai Patel University Modipuram, Meerut (India) and stored at 4ºC in refrigerator until use.

In vitro culture of mushroom

For in vitro culture of mushroom mycelium spawn culture method was used.

The wheat grains interwoven with mushroom mycelium (spawn) were aseptically transferred to malt agar media (malt extract-20.00g, peptone-10.00g, dextrose-10.00g, agar-20.00g. pH-5.5±0.2 and distilled water-1000.0 ml). The petri plates were incubated at 27ºC and were examined periodically over a week period. To facilitate rapid growth and mass cultivation mycelial discs from full plate growths were cut and placed onto malt agar plates

Mushroom (fruiting body as well as mycelium) metabolites were extracted for further antimicrobial investigation.

Extraction of metabolites from cultured mycelium

Pleurotus sapidus mycelium was grown on modified malt agar medium. Mycelial mat from 15 days old culture (mycelium) were used for extraction. Mycelium was removed and dried at 30°C in an oven for an hour. Dried mycelia were ground with pestle and mortar.1 gram ground mycelium material was extracted in 10 ml of each organic solvent (acetone and ethanol) and water (100 ˚C) separately. The extracts were kept on rotatory shaker for 48 hours. The extracts were then filtered over Whatman no.1 filter paper. The extracts were stored at 4ºC in refrigerator until use.

Extraction of metabolites from fruiting body

The fruiting bodies of mushroom were washed with turbulent flow of tap water in order to remove as much surface contamination as possible followed by washing with 70% ethyl alcohol. The washed and disinfected fruiting bodies of mushroom were dried in hot air oven at 30°C for 48 hours. Dried mushroom material was ground with a pestle and mortar. 1gram ground mushroom was extracted in 10 ml of organic solvents (acetone, ethanol, benzene, chloroform and ethyl acetate) and water separately. Extracts were kept in rotatory shaker for 48 hours. The extracts were filtered over Whatman no.1 filter paper. The extracts were stored at 4°C in refrigerator until use.

Collection of clinical samples

Different clinical samples were procured from diagnostic laboratories for isolation of microorganisms followed by their biochemical characterization and identification. Shri Ram Rai Laboratory (District Meerut, U.P.), Seth Laboratory (District Meerut, U.P.). All the collected samples were stored at 4ºC until further use.

Isolation, microscopic and biochemical characterization of microorganisms

The microorganisms were isolated from clinical samples by growing them on nutrient agar and selective media. They were further on characterized by microscopic and biochemical tests as gram staining, sugar fermentation, IMViC, catalase test, citrate utilization test, urease production test, mannitol fermentation and growth on EMB agar.

Resistance profile of microorganisms (Antibiogram)

Turbidity standard for inoculum preparation

To standardize the inoculums' density for a susceptibility test, a $BaSO_4$ 0.4 Turbidity

standard, equivalent to a 0.5 McFarland standard was prepared. The absorbance at 625nm was between 0.008 to 0.10. The barium sulfate suspension was transferred in 4 to 6mL aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculums. These tubes were tightly sealed and stored in the dark at room temperature.

Well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the inoculum was transferred into a tube containing 5 mL of a nutrient broth. The broths were incubated at 35 ºC until it achieved or exceeded the turbidity of the 0.5 Mc Farland standards. The turbidity of the actively growing culture was adjusted with sterile saline (0.85%) or broth to obtain turbidity optically comparable to that of the 0.5 Mc Farland standards, 100µL of inoculums suspension was spread with a sterile glass spreader on nutrient agar plates (Hi Media).

Antibiogram using Kirby bauer disc diffusion method

The Kirby Bauer disc assay was used for profiling the resistance patterns of microorganisms according to national committee for Clinical Laboratory standards Institute guidelines (Mulu *et al.,* 2017). Microbial broth was spread over Muller-Hinton agar plate and antibiotic discs were applied to the surface by using a dispenser and applying gentle pressure with sterile forceps to ensure complete contact of disc with agar. Plates were incubated for 24 hours at 35ºC for bacteria in a BOD incubator followed by zone measurement using Hi antibiotics zone scale^{TM} by resting plate's lid down on a black non-reflecting surface. The experiment was performed in triplicates. Plates were examined visually for isolated colonies within the inhibition zone that may have developed resistance.

In vitro antimicrobial activity of mushroom extract (mycelium as well as fruiting body)

Agar well diffusion method

To see the qualitative antimicrobial activity of natural mushroom extract and mycelium extract on pathogenic microorganism agar well diffusion method (an initial screening of antimicrobial activity) was used. Turbidity of activated culture of the test organism was visually adjusted using sterile saline solution to approximately that of 0.5 McFarland turbidity standards. On the surface of 90 mm nutrient agar plate was spread the test organism's broth using sterile spreader. The agar well diffusion assay was carried out by preparation of 4 wells of 5mm diameter using a sterile loop. The well was grouped as the test well and control wells. 80µl extracts (organic and aqueous) were filled into the well of agar plate separately by the help of micropipette. In control, 80µl of pure organic solvent and water were used separately in the control wells. Plates were incubated at 35ºC for 24 hours in BOD incubator. To score susceptibility, plates were rested lid down on a black non reflecting surface and the diameter of the inhibition zone was measured using Hi antibiotic zone scale^{TM}. The inhibition zones were recorded in the test as well as control wells. The above experiment was repeated thrice. The inhibition zones were compared with those of reference discs.

Results and Discussion

Cultivation of mushroom mycelium

Spawn culture method was employed to culture mushroom mycelium resulting in full plate growth in 15 days. The extracts

obtained after filtration were pale yellow in color.

Microscopy and biochemical tests of microorganisms under study

Biochemical test of microorganisms

Based on the microscopic and biochemical examination the cultures were identified as: Culture No.1 *Micrococcus*, Culture No. 2 *Enterobacter*, Culture No. 3 *Proteus*, Culture No. 4 *Staphylococcus aureus*, Culture No. 5 *Klebsiella*, Culture No. 6 *Pseudomonas*, Culture No. 7 *Bacillus*, Culture No. 8 *Escherichia coli*, Culture No. 9 *Enterobacter*, Culture No. 10 *Proteus* and Culture No. 11 *Staphylococcus aureus*. These cultures have to further go molecular characterization to complete their identification.

The antibiogram of experimental cultures was determined by disc diffusion method as shown in the Table.4.4.1. For Culture No.1 (*Micrococcus*) the lowest zone of inhibition was observed for tobramycin (5mm) and highest for gentamycin (25mm) hence resistant to five antibiotics. Culture No. 2 (*Enterobacter*) exhibits lowest zone of inhibition for vancomycin (8mm) and highest for ceftaxime (34mm). It is resistant to two antibiotics. For Culture No. 3 (*Proteus*) the lowest zone of inhibition was observed for ciprofloxacin (5mm) and highest for kanamycin (27mm). It shows resistance against two antibiotics. Culture No. 4 (*Staphylococcus aureus*) exhibited lowest zone of inhibition for vancomycin (11mm) and highest for ceftaxime (36mm), hence resistant for 4 antibiotics.

Culture No. 5 (*Klebsiella*) has shown resistance to all antibiotics. Culture No. 6 (*Pseudomonas*) is sensitive to ciplofloxacin (20 mm) and resistant to 5 antibiotics. Culture No. 7 (*Bacillus*) has shown sensitivity to ciplofloxacin and ofloxacin (20 mm) however it is resistant to 4 antibiotics. Culture No. 8 (*E. coli*) has shown resistance to three antibiotics.

Culture No. 9 (*Enterobacter*) has shown resistance to five antibiotics, being sensitive only to ciplofloxacin. Culture No. 10 (*Proteus*) has shown resistance to only one antibiotic with maximum zone of inhibition observed for olfloxacin (20 mm). Culture No. 11(*Staphylococcus aureus*) has shown no resistance to antibiotics used in study, highest zone of inhibition was observed in streptomycin (26 mm).

Antimicrobial screening of mushroom metabolites

It is apparent from the table that water extract of the mushroom possessed strong antibacterial activity which was most inhibitory against Culture No. 4 (*Staphylococcus aureus*) -25mm. The acetone extract was equally inhibitory against experimental cultures. Ethanol extract gives highest inhibition against Culture No. 1 and 4.

It is apparent from the table that water extract of the mycelium possesses strong antibacterial activity which was most inhibitory against Culture No. 4 (*Staphylococcus aureus*) -25mm. The acetone extract was equally inhibitory against experimental cultures. The ethanolic extract is most effective against Culture No. 4 (*Staphylococcus aureus*)-15 mm.

The highest zone of inhibition was demonstrated by benzene extract against Culture No. 11 (*S.aureus*) and Culture No. 8 (*E. coli*) showed the no zone of inhibition for four solvents.

Table.1 Microscopy and staining characteristics of microorganisms isolated from clinical samples

Culture	Morphology	Gram Staining
Culture No.1.	Cocci	Gram-positive
Culture No. 2	Rod	Gram-negative
Culture No. 3	Rod	Gram-negative
Culture No.4.	Cocci in grape like bunches	Gram-positive
Culture No.5	Rod	Gram-negative
Culture No.6	Rod	Gram-negative
Culture No.7	Rod	Gram-positive
Culture No.8.	Rod	Gram-negative
Culture No.9.	Rod	Gram-negative
Culture No.10.	Rod	Gram-negative

Table.2 Results of Biochemical test of microorganisms (Aneja, 2003)

Test Microorganism	Sugar Fermentation			Indole	MR/VP	Citrate	Urease	Catalase	MSA	EMB
	L	D	S							
C. No. 1	-	-	-	-	-/-	+	+	-	-	-
C.No. 2	AG	AG	AG	-	-/+	+	-	+	-	Purple colour
C. No. 3	-	AG	AG	+	+/-	-	+	+	-	Purple colour
C. No. 4	A	A	A	-	+/-	-	-	+	Color change of medium to yellow	-
C. No.5	AG	AG		-	-/+	+	+	+	-	Light purple colonies
C. No.6	-	-	-	-	+/-	+	-	+	-	Light color colonies
C. No.7	-	A	A	-	-/+	-	-	-	-	-
C. No.8	AG	AG	A	+	+/-	-	-	+	-	Metallic sheen
C. No.9	AG	AG	AG	-	-/+	+	-	+	-	Purple colour
C. No.10	-	AG	AG	+	+/-	-	+	+	-	Purple colour
C. No.11	A	A	A	-	+/-	-	-	+	Color change of medium to yellow	-

(C.No.: Culture number, A: Acid production, AG: Acid and gas production, +: presence or positive test, -:absence or negative test, MR: Methy red, VP: Voges Proskauer test, MSA: Mannitol Salt Agar, EMB: Eosin methylene blue agar)

Table.3 Antibiotic resistance profile of test microorganisms

Antibiotic (Quantities per disc)	C. No. 1		C. No. 2		C. No. 3		C. No. 4	
	ZI	A R P	ZI	A R P	ZI	A R P	ZI	A R P
Vancomycin (Va-30 µg)	6	R	8	R	24	S	11	R
Gentamycin (G-10 µg)	25	S	18	S	26	S	12	R
Streptomycin (S-10 µg)	12	I	24	S	23	S	26	I
Tobramycin (T-10 µg)	5	R	15	S	26	S	27	S
Ciprofloxacin (Cf-5 µg)	15	R	15	R	5	R	14	R
Ceftaxime (Cef-38 µg)	7	R	34	S	12	R	36	S
Kanamycin (K-30 µg)	6	R	27	S	27	S	12	R

(C.No. : Culture Number, ARP: Antibiotic resistance profile , ZI: Zone of inhibition (mm) , R Resistant:, I: Intermediate, S: Sensitive)

Table.4 Antibiotic resistance profile of test microorganisms

Antibiotic (Quantities per disc)	C. No. 5		C. No. 6		C. No. 7		C. No. 8	
	ZI	A R P	ZI	A R P	ZI	A R P	ZI	A R P
Streptomycin (10 µg)	-	R	6	R	-	R	20	S
Daflopristin (10 µg)	-	R	-	R	-	R	6	R
Tetracycline (30 µg)	-	R	6	R	-	R	20	S
Doxycline (30 µg)	-	R	6	R	-	R	20	S
Ciplofloxacin (5µg)	-	R	20	S	20	S	20	S
Ofloxacin (5 µg)	-	R	15	I	20	S	-	R
Nimuslide (10 µg)	6	R	-	R	13	I	6	R

C.No. : Culture Number, ARP: **Antibiotic resistance profile** , ZI: **Zone of inhibition (mm)** , R Resistant:, I: Intermediate, S: Sensitive

Table.5 Antibiotic resistance profile of test microorganisms

Antibiotic (Quantities per disc)	C. No. 9		C. No. 10		C. No. 11	
	ZI	A R P	ZI	A R P	ZI	A R P
Streptomycin (10 µg)	-	R	10	I	26	S
Daflopristin (10 µg)	-	R	10	I	12	I
Tetracycline (30 µg)	14	R	10	I	17	S
Doxycline (30 µg)	-	R	8	R	12	I
Ciplofloxacin (5µg)	20	S	15	S	18	S
Ofloxacin (5 µg)	18	I	20	S	16	S
Nimuslide (10 µg)	-	R	10	I	14	S

(C.No. : Culture Number, ARP: Antibiotic resistance profile , ZI: Zone of inhibition (mm)
R Resistant:, I: Intermediate, S: Sensitive)

Table.6 Antimicrobial effect of fruiting body extracts of *Pleurotus sajor*

S. No.	Test Microorganisms	Zone of inhibition (mm)					
		Acetone		Ethanol		Water	
		A_E	A_C	E_E	E_C	W_E	W_C
1.	Culture No. 1	10	-	15	-	20	-
2.	Culture No. 2	11	-	12	-	13	-
3.	Culture No. 3	9	-	10	-	11	-
4.	Culture No. 4	9	-	15	-	25	-

(- : no zone, A_C: Acetone control A_E: Acetone extract, E_{c-} ethanol control, E_E –Ethanol extract, W_{C-} water control, W_{E-} Water extract).

Table.7 Antimicrobial effect of mycelium extracts of *Pleurotus sapidus*

S. No.	Test Microorganisms	Zone of Inhibition (mm)					
		Acetone		Ethanol		Water	
		A_E	A_C	E_E	E_C	W_E	W_C
1.	Culture No. 1	10	-	12	-	14	-
2.	Culture No. 2	11	-	12	-	12	-
3.	Culture No. 3	9	-	10	-	11	-
4.	Culture No. 4	9	-	15	-	25	-

(- : no zone, A_C: Acetone control A_E: Acetone extract, E_{c-} ethanol control, E_E –Ethanol extract, W_{C-} water control, W_{E-} Water extract).

Table.8 Diameter of zone of inhibition (mm) of fruiting body extracts of *Pleurotus djamor*

S. No.	Microorganism	Diameter of zone of inhibition (mm) of different extracts									
		Ethyl acetate		Ethanol		Benzene		Chloroform		Water	
		EA_E	EAc	E_E	E_C	B_E	B_C	C_E	C_C	W_E	W_C
1	Culture No. 5	6	6	4	-	6	4	6	-	5	-
2	Culture No. 6	5	5	-	-	6	5	-	-	6	-
3	Culture No. 7	9	6	-	-	-	-	-	-	-	-
4	Culture No. 8	6	-	-	-	-	-	-	-	-	-
5	Culture No. 9	6	6	6	-	6	-	8	-	9	-
6	Culture No. 10	6	6	-	-	5	-	-	5	-	-
7	Culture No. 11	5	-	8	-	15	-	-	-	6	-

(- : no zone, EA_c- ethylacetate control, EA_{E-} Ethyl acetate extract, E_{c-} ethanol control, E_E –Ethanol extract, C_{c-} choloroform control, C_{E-} choloroform extract, B_{C-} benzene control, B_{E-} Benzene extract, W_{C-} water control, W_{E-} Water extract).

Table.9 Diameter of zone of inhibition (mm) of fruiting body extracts of *Pleurotus florida*

S.No.	Microorganism	Diameter of zone of inhibition (mm) of different extracts									
		Ethyl acetate		Ethanol		Benzene		Chloroform		Water	
		EA_E	EA_C	E_E	E_C	B_E	B_C	C_E	C_C	W_E	W_c
1	Culture No. 5	6	6	-	-	6	-	9	6	-	-
2	Culture No. 6	5	-	-	-	-	-	-	-	-	-
3	Culture No. 7	4	-	-	-	-	-	-	-	-	-
4	Culture No. 8	5	5	6	6	-	-	-	-	-	-
5	Culture No. 9	15	-	10	-	9	-	8	-	-	-
6	Culture No. 10	6	6	6	6	-	-	6	6	-	-
7	Culture No. 11	14	6	11	-	8	-	9	-	-	-

(- : no zone, EA_c- ethylacetate control, EA_{E-} Ethyl acetate extract, E_{c-} ethanol control, E_E –Ethanol extract, C_{c-} choloroform control, C_{E-} choloroform extract, B_{C-} benzene control, B_{E-} Benzene extract, W_{C-} water control, W_{E-} Water extract).

Photographic plate.1

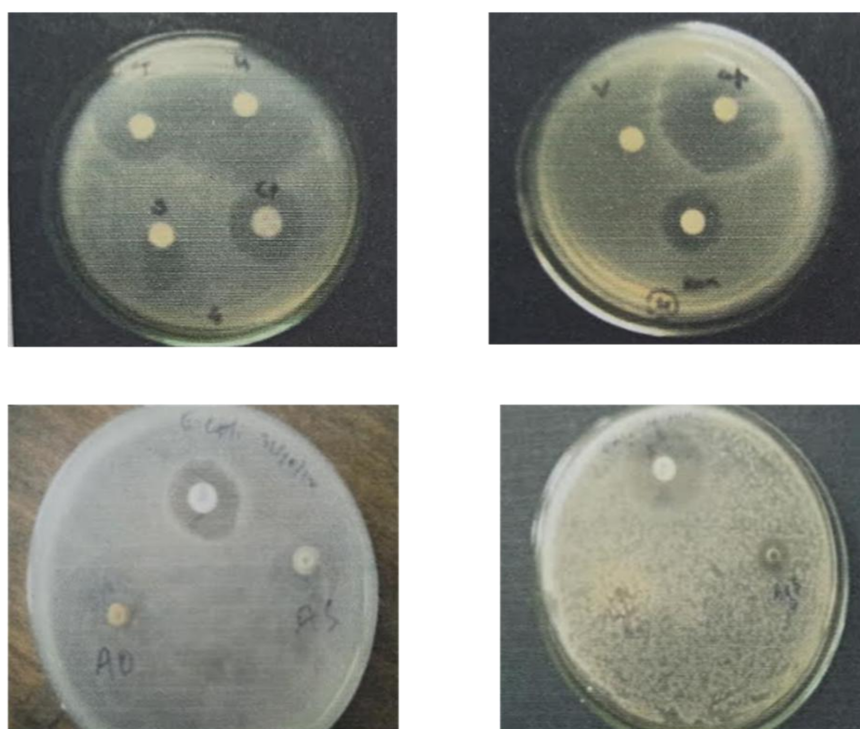
Stages of cultivation of mushroom mycelium and extraction of bioactive metabolites from fruiting bodies of *Pleurotus sp* and *Agaricus bisporus*

Table.10 Diameter of zone of inhibition (mm) of fruiting body extracts of *Agaricus bisporus*

S. No.	Microorganism	Diameter of zone of inhibition (mm) of different extracts									
		Ethyl acetate		Ethanol		Chloroform		Benzene		Water	
		EA_E	EA_c	E_E	E_c	C_E	C_C	B_E	B_C	W_E	W_C
1	Culture No. 5	5	6	6	5	-	-	-	-	-	-
2	Culture No. 6	-	-	6	6	-	-	5	-	-	-
3	Culture No. 7	-	5	9	7	5	-	6	-	5	-
4	Culture No. 8	6	-	6	6	6	4	-	-	-	-
5	Culture No. 9	14	-	8	-	15	-	20	-	15	-
6	Culture No. 10	10	-	9	8	-	-	7	-	-	-
7	Culture No. 11	16	-	9	-	20	-	14	-	24	-

(- : no zone, EA_c- ethylacetate control, EA_{E}- Ethyl acetate extract, E_c- ethanol control, E_E –Ethanol extract, C_c – choloroform control, C_E- choloroform extract, B_C- benzene control, B_E- Benzene extract, W_C- water control, W_E - Water extract).

Photographic plate.2



Antibiogram of pathogenic microorganisms and antibiotic activity of bioactive metabolites

The highest zone of inhibition was demonstrated by ethyl acetate extract against Culture No. 9 (*Enterobacter*)- 15mm and the lowest zone of inhibition were demonstrated by Culture No. 6 (*Pseudomonas*) and Culture No. 7 (*Bacillus*). The highest zone of inhibition was demonstrated by water extract against Culture No. 11 (*S.aureus*) and Culture No. 6 (*Pseudomonas*) showed the lowest zone of inhibition for all solvents.

Implication

The microorganisms isolated from clinical samples have revealed their resistance profiles against different antibiotics be it aminoglycosides, macrolides or fluoroquinolones. This indicates as alarming trend which may cripple the healthcare industry if alternatives are not developed.

The present study has further revealed the antimicrobial activity of the metabolite of the macrofungus extracted with different solvents.

All the extracts form species of *Pleurotus* and *Agaricus* were observed to inhibit the growth of both gram negative and gram positive pathogenic microorganisms as shown by their respective zones of inhibition, thus suggesting their broad-spectrum and antimicrobial activity. The zone of inhibition obtained is comparable to commercially available antibiotics many of which are second and third line of antibiotics. The observed differences in the susceptibilities of microorganisms tested with the solvent extracts provides an indication that the solvents used have varying abilities to extract bioactive substances from mushrooms under study.

The study thus gives a viewpoint that the bioactive metabolites isolated from mushrooms hold immense potential to be further explored as an antimicrobial agent. Both fruiting body and mycelium extracts have shown their effectiveness against microorganisms therefore the need arises to further investigate the bioactive compounds and their mode of action.

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