WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 16, 550-564. Research Article ISSN 2277–7105

STUDY OF ANTIBACTERIAL AND PHYTOCHEMICAL PROPERTIES OF WHITE-ROT FUNGI COLLECTED FROM THE NORTH-WESTERN REGIONS OF HIMACHAL PRADESH.

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Article Received on
14 October 2017,
Revised on 03 Nov. 2017,
Accepted on 24 Nov. 2017
DOI: 10.20959/wjpr201716-10134

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ABSTRACT

Mushrooms have been recognized as functional foods and a source for the development of medicines and nutraceuticals. In present study, methanol and hot water extract of the all six mushroom cultures viz: *Morchella* sp., *Mycena* sp., *Trametes* sp., *Agaricus* sp., *Chlorophyllum* sp., *Schizophyllum* sp. showed good antimicrobial activity against pathogenic bacteria named as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia. coli, Salmonella typhi, Klebsiella. pneumoniae.* Among all the cultures, the methanolic extract of the *Agaricus* sp. showed maximum activity (36±1.4) followed by *Chlorophyllum* sp. (35±1.4) against *K. pneumonia* and least activity showed by *Trametes* sp. (15±1.4) against *E. coli* followed by *Morchella* sp. (16.5±0.7) against *P. aeruginosa.* Among the entire Mushroom cultures the hot water extract of *Chlorophyllum* sp. showed maximum activity (37±1.4) against *K. pneumonia* followed by *Agaricus* sp. (36±1.4) against *P. aeruginosa* and least activity showed by *Mycena* sp. (15.5±0.7) and *Morchella* sp. (16.5±2.1) against *S. aureus.* The results obtained revealed the presence of alkaloids, carbohydrates, flavonoids, terpenoids, phenolic compounds and tannins. The hot water extract of *Chlorophyllum* sp. (7.77±0.9) and *Agaricus* sp. (7.66±0.7) showed significantly high ($P<0.05$) phenolic content followed by *Schizophyllum* sp. (5.14±1.3). *Schizophyllum* sp. (17.94±13.1) consist of flavonoids compounds in high amount followed by *Agaricus* sp. (12.99±1.2) and *Chlorophyllum* sp. (10.68±2.0). It therefore suggested from the present study that mushrooms selected for the study could be potential source of compounds with beneficial biological activities and new drug discovery.

KEYWORDS: Mushrooms, antibacterial activity, MIC, Phytochemicals properties.

INTRODUCTION

Mushrooms are fleshy fungi, fruiting bodies bear spores which typically generated above ground on soil or on their food sources which forms a major group of smaller plant kingdom. Mushroom also known as fruiting body of the gill fungi. Mushrooms are large enough to seen by naked eyes. Mushrooms have distinctive fruiting body. Because of the nutritive contents, some mushrooms are eatable while some are utilized extensively in traditional medicines (Karaman *et al*., 2012). The utilization of mushrooms as food is very old. People have harvested mushrooms from the wild for medicine and food for thousands of years. There are about 1200 species of mushroom utilized in 85 dissimilar countries for their medicinal properties and a total of 126 therapeutic functions are thought to be generated by them (Wasser, 2011). Mushroom cultivation is very applicative in the appliance against diminishing quality of human health, environmental pollution and shortage of food, which human being still face and keep on to face due to the continued rise of the world population, natural resource degradation and impression from climate change (Oseni *et al*., 2012). Species of mushroom which are eatable are elevated nutritive and also compared with milk, eggs and meat and they reveal a constitution in amino acids similar to animal proteins (Longvah and Deosthale, 1998). Except the nutritional characteristics, a great body of information specifies that mushroom have been also considerably studied for their medicinal properties especially due to their richness in biologically effective compounds that presented antioxidant, antimicrobial properties, strengthening the immune system and assuring against carcinogens. Biologically, Basidiomycetes exhibit a broad spectrum of pharmacological activities, showing antimicrobial, antioxidant, anti-inflammatory, antitumor and immunomodulating effects and demonstrating an important impact on cardiovascular disorders and diabetes (Gunde-Cimerman, 1999; Wasser and Weis, 1999).

Antimicrobial compounds generated by algae and fungi (Mushrooms) against pathogens have received considerable attention as a new source of unique antimicrobial substances (Ramesh and Pattar, 2010; Bhagvathy *et al*., 2011). A few studies were reported on the antimicrobial activities of some eatable mushrooms and their bioactive compounds. In *Laetiporus sulphureus* antioxidant and antimicrobial activities were reported and co-related to the phenols and the flavonoids contents (Turkoglu *et al*., 2006) and guanine sesquiterpenoids isolated from the fruit bodies of eatable *Lactarius* species proved to have antibacterial

activity (Anke *et al*., 1989). Bioactive compounds having the tendency to scavenge free radicals and therefore provide defense against the disease. It is another point of importance when searching for the unique bioactive compounds from organism and its ability to generate secondary metabolites (Donadio *et al*., 2002). Other bioactive substances such as triterpenes, lipids and phenols have also been identified and characterized in mushroom with medicinal properties (Maiti *et al*., 2008). Carbohydrate, like polysaccharides present in the cell wall of fungus. Polysaccharides provide excessive variety of bioactivities. Several bioactive compounds such as polysaccharides, polysaccharides-peptides, nucleosides and triterphenol have been identified in number of species mushroom species (Wasser and Weis, 1999). Mushroom also contain vitamins A and C of β-carotene and a great variety of secondary metabolites such as phenolics compounds, polyketides, terpenes, steroids and phenols, entire have protective affects because of their antioxidant properties (Jaykumar *et al*., 2009; Soares *et al*., 2009).

MATERIALS AND METHODS

Samples of wild mushrooms were procured from Mycology Research Laboratory, Shoolini University, Solan. The samples were maintained and subculture monthly on Malt Extract Agar. Sub culturing for pure tissue mycelial production was prepared by transferring a small bit of 5mm from the mother plate onto fresh solid media plates. Five pathogenic microorganisms namely *Klebsiella pneumoniae, Salmonella typhi, Streptococcus aureus, Escherichia coli, Pseudomonas aeruginosa* were procured from the Parasitology Research Laboratory, Shoolini University, Solan. Strains were sub cultured and maintained on nutrient agar slants. Loop-full of bacterial cultures were streaked on their respective medium and incubated (bacteria at 37±1ºC). These cultures were then stored under refrigeration. Mycelial morphology (color, density and type of growth) microscopic features (clamp connections, mycelia type) were examined using Congo red as a stain.

Preparation of extracts

The methanolic extract of mushrooms was prepared by weighing accurately freeze-dried mycelial biomass (5g) into a 100 ml sterilin and shaken overnight (18–24 hours) with 50 ml of methanol at room temperature. The extract was then filter through Whatman paper No.1. The residue was then resuspended in another 50 ml of methanol. The process was repeated twice. The combined methanol filtrate was transferred to a pre-weighted sterilin and the solvent was evaporated by applying a constant flow of air. Residual methanol was removed

by vacuum pressure at 50°C overnight in an oven and the dry weight recorded. The hot water extract was prepared by weighing accurately dry mycelial biomass and 10 ml deionize water was added to it. The mixture was heated at 100°C for 20 minutes and allowed to cool for 20 minutes. After this, the filtrate was separated from the mycelial biomass by using No.42 Whatman filter paper. The process was repeated twice. The combined filtrate was then freeze-dried and the dry weight of sample was recorded (Bains and Tripathi, 2015).

Anti-bacterial activity

Entire bacterial strains were incubated at 37°C for 24 hours. Inoculums were prepared by transferring the colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standards having approximately 10^8cfu/ml for bacteria. Mueller - Hinton Agar (Merck) medium was utilized (Hammer *et al*., 1999). Muller Hinton agar plates with 4% NaCl supplementation were prepared. Sterilized swabs were dipped in standardized bacterial suspension with inoculums size of 1.5 X 10^8cfu/ml prepared above and excess culture was removed by turning the swab against the side of the tube. Inoculums were spread evenly over the entire surface of Muller Hinton Agar plates. These plates were allowed to dry for at least 15 min and then well (7mm diameter) were made on petridish using sterile cork borer. About 25µl extract was introduced into bore agar wells using sterile dropping pipette. These plates were kept inside the refrigerator at 4°C for 6 hours to allow proper diffusion of extracts into medium. The plates were then examined for antibacterial activities of extracts after 24 hours of incubation at 37°C. Antibacterial activity was determined by measuring the diameter zone of inhibitions in mm (Moshi *et al*., 2006).

Determination of Minimal Inhibitory concentration for estimating Antibacterial activity

The resazurin solution was prepared by dissolving 270 mg of resazurin powder (Sigma-Aldrich GmbH, Germany) in 40 ml sterile distilled water. Each well of 96-well microtitre plate was aliquot with 50 µl of Muller Hinton Broth (MHB); 12th well (sterility control) was added with 100 µl of Ciprofloxacin antibiotic as positive control. 11th well (growth control) was added with MHB with 10% DMSO. 50 µl of mycelial extract initially dissolved in 10% DMSO to the concentration of 100 mg/ml was added into the first well and a serial 2-fold dilution was performed by transferring 50 µl of the suspension to the subsequent wells up till the 10th well; the final 50 µl of the suspension was discarded. Then, of 5 µl bacterial suspension was added to each well and incubated at 37ºC for 24 hours. After 24 hours of incubation 5 µl of resazurin was added to each well. Plates were incubated at 37ºC for

additional 1 hour. After one hour of incubation the plates were read for color change from blue to purple/ pink. A blue colored solution indicated the growth restriction in the test wells, while pink to colorless solution indicated microbial growth or absence of restriction (Wiegand *et al.*, 2008).

Determination of Phytochemical compounds

Determination of phytochemical compounds was done for three hot water extracts. The freshly prepared hot water extracts were subjected to phytochemical analysis in order to ensure the existence of phytochemical constituents. Mayer's test and Wagner's test were done for the identification of alkaloids. Molisch's test and Benedict's test were done for the identification of carbohydrates. Alkaline reagent test was done to check the presence of flavonoids. Salkowski test was performed to check the presence of terpeonoids. Ferric chloride test and Lead acetate test was done to identified the presence of phenolic compounds and tannins respectively (Bains and Tripathi, 2016).

Test for detection of glycosides and saponins

To the 2ml of extract, 1 ml of glacial acetic acid and 5% of ferric chloride was be added. Then few drops of concentrated sulphuric acid were added. Greenish blue color indicates the positive results for glycosides. To the 1 ml of extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. Length-wise formation of 1 cm layer of foam indicates the presence of saponins (Chaudhary and Tripathi, 2015).

Estimation of Phytochemical compounds

Phenolic Compound estimation was done by taking 100µl of entire extract of mushroom samples and mixed with 1.5ml of Folin-Ciocalteu reagent and incubated at room temperature for one minute followed by the addition of 1.5ml sodium carbonate (60g/l). The tubes were then vortex-mixed for 15s and allowed to stand for 90 minutes in dark at room temperature. Absorbance was then measured at 725 nm. Gallic acid was be utilized as standard curve with concentration ranges from 1 to 100µg/ml (R^2=0.996) and the results were expressed as mg gallic acid equivalent (GAE)/g. Entire experiments were performed in duplicates (Singleton *et al.*, 1999). Flavonoid contents was measured by taking 250 µl extract of entire extract of mushroom samples and mixed with 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. 150 µl of 10% $AlCl_3.H_2O$ of was be added after 5 min. Then 275 µl of distilled water and 500 μl of 1 M NaOH would be added to the mixture after 6 min. The solution was then mixed well and the intensity of pink color was measured at 510 nm. The flavonoid

compounds estimation would be carried out in triplicate. Quercetin was utilized for calibration curve with concentration range of 20-100 µg/ml (R^2=0.9938). The results would be mean values ± standard deviations and expressed as milligrams of (+) quercetin equivalents (QEs) per gram of extract (Jia *et al.*, 1999).

β-carotene and lycopene determination

The dried entire three extracts of each mushroom samples (100 mg) would be vigorously shake with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No.4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (Nagata and Yamashita, 1992). Content of β-carotene and lycopene were calculated by using following equations.

Lycopene (mg/100mg) = -0.0458 A_{663}+ 0.372 A_{505} -0.0806 A_{453}

β-carotene (mg/100mg) = 0.216A_{663}-0.304 A_{505}+ 0.452 A_{453}

RESULTS AND DISCUSSIONS

Screening by Antibacterial activity

Antibacterial effect of the methanolic and hot water extract of all six mushroom cultures were studied against five pathogenic bacteria i.e. *Salmonella typhi, Streptococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*. Ciprofloxacin antibiotic taken as positive control and DMSO was taken as negative control. All the methanolic and hot water extract of cultures were found to be active against all the five pathogenic bacteria. Among all the cultures, the methanolic extract of the *Agaricus* sp. showed maximum activity (36±1.4) against *K. pneumonia* followed by *Chlorophyllum* sp. (33±2.8) against *S. typhi* followed by *Schizophyllum* sp. (30±1.4) against *E. coli* followed by *Trametes* sp. (27±1.4) against *K. pneumonia* followed by *Mycena* sp. (17.5±0.7) against *S. typhi* followed by *Morchella* sp. (16.5±0.7) against *E. coli* and least activity showed by *Trametes* sp. (15±1.4) against *E. coli* followed by *Morchella* sp. (16.5±0.7) against *P. aeruginosa* followed by *Mycena* sp. (17.5±0.7) against *S. aureus* followed by *Agaricus* sp. and *Schizophyllum* sp. (20.5±0.7) against *S. aureus.* Among the entire Mushroom cultures the hot water extract of *Chlorophyllum* sp. showed maximum activity (37±1.4) against *K. pneumonia* followed by *Agaricus* sp. (36±1.4) against *P. aeruginosa* followed by *Schizophyllum* sp. (33±2.8) against *E. coli* followed by *Mycena* sp. (29.5±2.1) against *E. coli* followed by *Morchella* sp. and *Trametes* sp. (27.5±2.1) against *P. aeruginosa* and least activity showed by *Mycena* sp. (15.5±0.7) and *Morchella* sp. (16.5±2.1) against *S. aureus* followed by *Morchella* sp.

(24±2.8) against *E. coli* followed by *Trametes* sp. (25±1.4) against *K. pneumonia* followed by *Chlorophyllum* sp. (28.5±2.1) against *E. coli* followed by *Schizophyllum* sp. (29.5±2.1) against *P. aeruginosa.* The results of antibacterial effect of methanolic and hot water extract of different species are shown in Table-1; Fig.1.

The antimicrobial properties of *Agaricus blazei* have been confirmed in most research studies using *in vitro* methods (Osaki *et al.,* 1994; Mazzutti *et al.,* 2012; Stojkovic *et al.,* 2014; Sokovic *et al.,* 2014) and *in vivo* testing mice, noting that in this sample group the mushroom extract exerted action against microorganisms that cause lethal infections caused by *Pneumococci* (Benardshaw *et al.,* 2005) and fecal peritonitis (Benardshaw *et al.,* 2006). The results for research which confirmed the antimicrobial activity of extracts of the mushroom *Agaricus blazei* mostly identified greater inhibition for the Gram+ve bacteria (compared with Gram-ve bacteria), such as *Salmonella typhi*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Staphylococcus aureus* and coliforms (Koneman *et al.,* 1999). Many species of fungi have been known to demonstrate a range of antimicrobial properties. For this reason, the aim of the present study was to perform an assessment of the antimicrobial potential of six samples of fungal species against highly relevant pathogens including five bacteria. In the present study, the methanolic extract of *Agaricus* sp. showed maximum antibacterial activity (36±1.4) against *Klebsiella pneumoniae* and hot water extract of *Agaricus* sp. showed maximum antimicrobial activity (36±1.4) against *Pseudomonas aeruginosa.*

Table 1: Antibacterial activity of methanol extract (M) and hot water extract (A) of different mushroom cultures against the different pathogenic bacteria.

S. No	Isolates	Zone inhibition diameter in mm									
		S. aureus		*P. aeruginosa*		*E. coli*		*S. typhii*		*K. pneumonia*	
		M	A	M	A	M	A	M	A	M	A
1	***Morchella* sp.**	18.5 ± 0.7^{bc}	16.5 ± 2.1^{bc}	16.5 ± 0.7^{b}	27.5 ± 2.1^{b}	22 ± 1.4^{cd}	24 ± 2.8^{b}	21 ± 1.4^{bc}	27.5 ± 0.7^{bc}	18.5 ± 0.7^{e}	26 ± 1.4^{bc}
2	***Mycena* sp.**	17.5 ± 0.7^{c}	15.5 ± 0.7^{c}	18 ± 1.4^{b}	25.5 ± 2.1^{b}	21.5 ± 2.1^{d}	29.5 ± 2.1^{ab}	25 ± 1.4^{b}	26 ± 1.4^{c}	23.5 ± 3.5^{de}	26.5 ± 2.1^{bc}
3	***Trametes* sp.**	16.5 ± 2.1^{c}	20.5 ± 0.7^{b}	19.5 ± 0.7^{b}	27.5 ± 0.7^{b}	15 ± 1.4^{e}	26.5 ± 2.1^{b}	18 ± 1.4^{c}	27.5 ± 2.1^{bc}	27 ± 1.4^{cd}	25 ± 1.4^{c}
4	***Agaricus* sp.**	20.5 ± 0.7^{ab}	34.5 ± 2.1^{a}	23.5 ± 0.7^{a}	36 ± 1.4^{a}	27 ± 1.4^{ab}	29.5 ± 2.1^{ab}	33 ± 1.4^{a}	31.5 ± 2.1^{ab}	36 ± 1.4^{a}	32.5 ± 2.1^{abc}
5	***Chlorophyllum* sp.**	22 ± 1.4^{a}	32.5 ± 2.1^{a}	23 ± 1.4^{a}	35 ± 1.4^{a}	25.5 ± 0.7^{bc}	28.5 ± 2.1^{ab}	33 ± 2.8^{a}	32.5 ± 0.7^{a}	34.5 ± 2.1^{ab}	37 ± 1.4^{a}
6	***Schizophyllum* sp.**	20.5 ± 0.7^{ab}	31.5 ± 0.7^{a}	18.5 ± 0.7^{a}	29.5 ± 2.1^{b}	30 ± 1.4^{a}	33 ± 2.8^{a}	32 ± 1.4^{a}	32.5 ± 2.1^{a}	29.5 ± 2.1^{bc}	36 ± 1.4^{ab}
7	**+ve control** (Ciprofloxacin)	38	38	40	40	39	39	38	38	42	42

Note: In each column different letters means significant difference at $p<0.05$. Values are Mean ±SD (n=2).

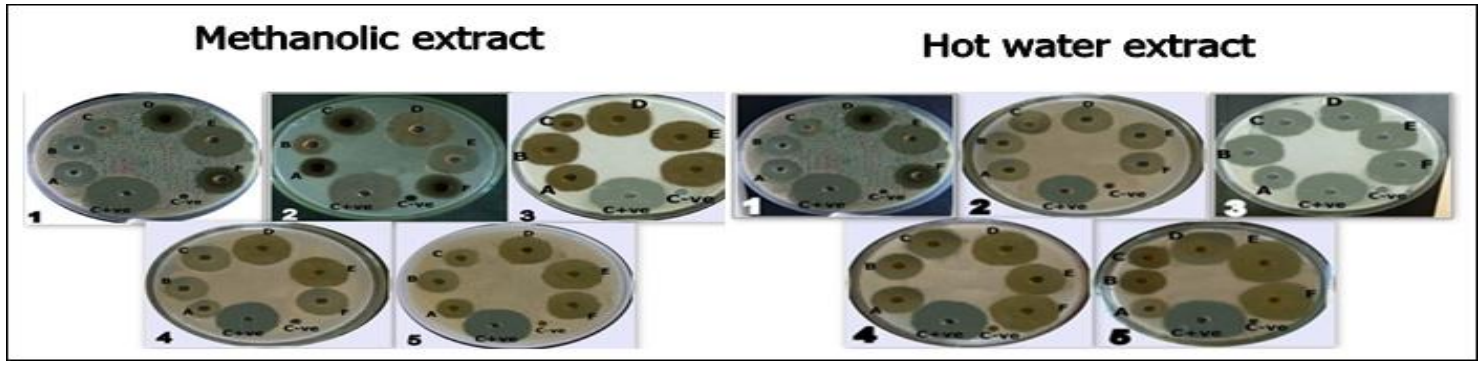

Fig.1: Zone of inhibition of Methanolic extract and hot water extract of six mushroom cultures against (1) *S.aureus.* (2) *P. aeruginosa* (3) *E. coli* (4) *S. typhi* (5) *K. pneumonia.*

Activity of extract of (A) *Morchella* sp., (B) *Mycena* sp., (C) *Trametes* sp., (D) *Agaricus* sp., (E) *Chlorophyllum* sp., (F) *Schizophyllum* sp. C+ve is positive control Ciprofloxacin and C-ve is negative control DMSO.

Minimal inhibitory concentration of methanolic and hot water extract

Result has shown all methanol and hot water extract of all six mushroom cultures exhibit a good antibacterial spectrum activity. The methanol extract of *Agaricus* sp., *Schizophyllum* sp., was best to potentiate the antibacterial activity of *K. pneumoniae* with MIC 0.78 mg/ml. against *P. aeruginosa* followed by *Agaricus* sp., *Chlorophyllum* sp., showed good antibacterial activity with MIC 1.56 mg/ml followed by *Chlorophyllum* sp. and *Schizophyllum* sp. showed high antibacterial potential against *E. coli* with MIC 3.12 mg/ml. Hot water extract of *Mycena* sp. and *Schizophyllum* sp. showed good antimicrobial potential against *S. aureus* with MIC 0.78 mg/ml against *K. pneumonia* followed by extract of *Trametes* sp. and *Agaricus* sp. showed good antibacterial with MIC 1.56 mg/ml each against *K. pneumonia*. The results of minimal inhibitory concentration of methanolic and hot water extract were shown in Table-2.

Table 2: Minimal inhibitory concentrations of methanol and hot water extracts of mushroom cultures against pathogenic bacteria. Here M stands for Methanolic extract and A for hot water extract.

Sr No	Isolates	Minimal inhibitory concentration (mg/ml)									
		S. aureus		*P. aeruginosa*		*E. coli*		*S. typhii*		*K.pneumonia*	
		M	A	M	A	M	A	M	A	M	A
1	***Morchella* sp.**	12.5	6.25	12.5	1.56	12.5	6.25	25	1.56	12.5	12.5
2	***Mycena* sp.**	3.12	0.78	12.5	3.12	3.12	0.78	12.5	25	1.56	3.12
3	***Trametes* sp.**	1.56	3.12	12.5	12.5	3.12	3.12	6.25	12.5	3.12	1.56
4	***Agaricus* sp.**	6.25	3.12	1.56	12.5	1.56	3.12	3.12	12.5	0.78	1.56
5	***Chlorophyllum* sp.**	1.56	3.12	1.56	6.25	3.12	6.25	12.5	0.78	6.25	3.12
6	***Schizophyllum* sp.**	6.25	0.78	3.125	0.78	3.12	6.25	6.25	3.125	0.78	3.12

Preliminary phytoconstituents analysis

On the basis of initial screening, three mushroom cultures have been selected for phytochemical analysis. The preliminary phytoconstituents analysis of all three isolates revealed that all the three extracts contain Tannins, Phenols and Saponins. Glycosides were absent in *Agaricus* sp. and *Schizophyllum* sp. and alkaloids, carbohydrates and flavonoides were absent in *Chlorophyllum* (Table-3).

Table 3: Phyto-chemical analysis of hot water extracts of three isolates.

Sr. No	Isolates	Alkaloids	Tannins	Terpenoids	Carbohydrates	Phenols	Flavonoids	Glycosides	Saponins
1	***Agaricus* sp.**	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
2	***Chlorophyllum* sp.**	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve
3	***Schizophyllum* sp.**	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Quantitative analysis of phytochemicals

The quantitative analysis of phytochemical revealed that *Agaricus* sp., *Chlorophyllum* sp., and *Schizophyllum* sp. showed the presence of phenols, flavonoids, β-carotene and lycopene present in the above in specific quantities were shown in table 4. The maximum amount of phenols were found to be present in the *Chlorophyllum* sp. (7.77±0.92) followed by *Agaricus* sp. (7.66±0.77) and *Schizophyllum* sp. (5.14±1.39). Flavonoids were found to be present in *Schizophyllum* sp. (17.94±13.16) in high amount followed by *Agaricus* sp. (12.99±1.24) and *Chlorophyllum* sp. (0.68±0.2). β-carotene were found to be present in maximum amount in *Chlorophyllum* sp. (5.60±1.15) followed by *Schizophyllum* sp. (5.09±1.86) and *Agaricus* sp. (5.05±0.37) and lycopene was present in maximum amount in *Agaricus* sp. (3.89±0.16) followed by *Schizophyllum* sp. (3.17±3.02) and *Chlorophyllum* sp. (3.17±3.02). Results of phytochemicals are shown in Table-4; Fig.2.

The phytochemical analysis reveals that the extracts were rich source of phytoconstituents containing carbohydrate, saponins, phenols, glycosides, terpenoids, flavanoids. The extract could be potential source of new antimicrobial agents and scientifically validates the use of the macrofungi in traditional medicine. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti inflammatory and anti- cancer effects among others. Phenolic compounds are antioxidant and exhibit a wide range of spectrum medicinal properties such as anti cancer and anti inflammatory. These mushrooms can be therefore being harnessed in the management of oxidative stress induced disease since phenol and flavanoids have been shown to posses various antioxidant functions (Harborne *et al.*, 2005). Saponins comprise a large family of structurally related compounds containing a steroid or triterpernoid aglycone. They are reported to have a wide range of pharmacological properties that exert various benefits, such as anti-inflammatory and anti-diabetic properties (Lee *et al.*, 2012). Terpenoids (isoprenoids) are secondary metabolites with molecular structures containing carbon backbones made up of isoprene. The compounds have been

reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti- cancer among others (Rosalin *et al.*, 2011).

Table 4: Bioactive compounds present in hot water extract.

Estimation of bioactive compounds in hot water extract					
S.No	**Isolates**	**Phenols (mg/g)**	**Flavonoid (mg/g)**	**β-carotene (µg/g)**	**Lycopene (µg/g)**
1	***Agaricus* sp.**	7.66 ± 0.77^{a}	12.99 ± 1.2^{b}	5.05 ± 0.37^{a}	3.89 ± 0.16^{a}
2	***Chlorophyllum* sp.**	7.77 ± 0.92^{a}	0.68 ± 0.2^{c}	5.60 ± 1.15^{a}	2.57 ± 0.66^{b}
3	***Schizophyllum* sp.**	5.14 ± 1.39^{b}	17.94 ± 13.16^{a}	5.09 ± 1.86^{a}	3.17 ± 3.02^{ab}

In each column different letters means significant difference at $p<0.05$. Values are Mean± SD (n=2).

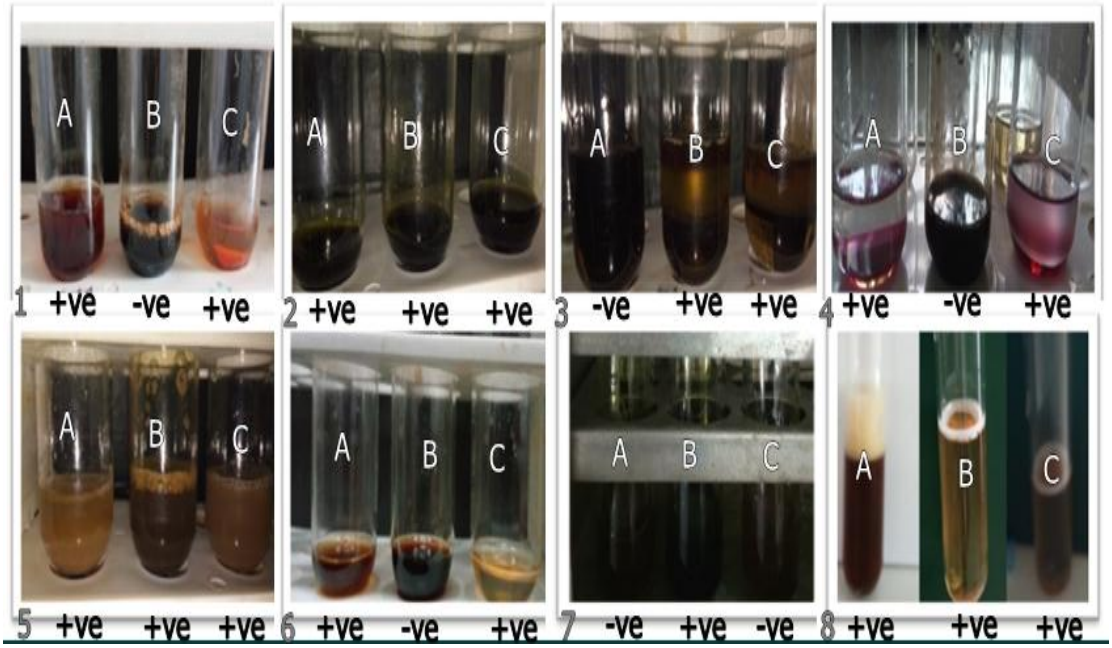

Fig.2: Results of (1) Alkaloids (2) Tanins (3) Terpenoids (4) Carbohydrates (5) Phenols (6) Flavonoids (7) Glycosides and (8) Saponins (A) *Agaricus* sp., (B) *Chlorophyllum* sp., (C) *Schizophyllum* sp.

SUMMARY AND CONCLUSION

Mushrooms are rich source of natural antibiotics, in these, the cell wall glucans are well known for their immunomodulatory properties and many of the externalized secondary metabolites. The aim of present investigation was to study the antimicrobial effect from six different mycelial cultures of mushrooms and estimation of bioactive compounds from three different mycelial cultures of mushrooms. It observed upon screening that all the six different wild mushrooms mycelial extract, showed good antibacterial activity. The methanol extract

of *Agaricus* sp. shows maximum activity (36±1.4) against *K. pneumonia*, where as *Morchella* sp. shows least activity (18.5±0.7) against *K. pneumonia*. Among the entire Mushroom cultures the hot water extract of *Chlorophyllum* sp. showed maximum activity (37±1.4) against *K. pneumonia* and *Trametes* sp. showed least activity (25±1.4). According to results all six methanol extract of mushroom cultures exhibit a good antibacterial spectrum activity. The methanol extract of *Agaricus* sp., *Schizophyllum* sp., was best to potentiate the antibacterial activity of *K. pneumonia* with MIC 0.78 mg/ml. Against *P. aeruginosa* extract of *Agaricus* sp., *Chlorophyllum* sp., showed good activity with MIC 1.56 mg/ml where as the Hot water extract of *Mycena* sp. and *Schizophyllum* sp. showed good antimicrobial potential against *S. aureus* with MIC 0.78 mg/ml. Against *K. pneumonia* extract of *Trametes* sp. and *Agaricus* sp. showed good antimicrobial with MIC 1.56 mg/ml each. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, terpenoids, glycosides, saponins and tannins. Further estimation of phenolic compounds phenol, flavonoids and carotene was done. Among all three extracts of mushroom cultures taken for study, the hot water extract of *Agaricus* sp. consist of phenolic compounds in high amount (7.66±0.77) and *Schizophyllum* sp. consist of flavonoids compounds in high amount (17.94±13.16). The information therefore collected in the present study considered useful for further development of the selected mushrooms cultivation process on industrial scale, particularly to enhance the production of bioactive metabolites. The six isolates showed to possess strong antimicrobial activity and out of the six different isolates, only three were selected for the presence of the bioactive compounds present in it and all the three isolates showed good results for the presence of bioactive compounds. Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity. The presence of some compounds, such as ascorbic acid, α-tocopherol, β-carotene and phenolic compounds is an indication that mushrooms are equipped with antioxidant properties. Of these, flavonoids and phenolic acids contribute largely to the antioxidant affect. The main antioxidants present in mushroom are phenolic acids and flavonoids, carotenoids, tocopherol and ascorbic acid that are important protective agents for human health. The present findings suggest that there is a potential to use the fungal components from the analyzed isolates to act as antioxidants and antimicrobial in food, which would enhance their quality and nutritive value.

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