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Studies on evaluation of antibacterial activities of some cultivated mushrooms against human pathogenic bacteria

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ABSTRACT

Mushrooms have been used as source of nutritive food and medicine since ancient time. In this study, the antibacterial activity of acetone, aqueous and methanol extracts of *Agaricus bisporus*, *Calocybe indica*, *Flammulina velutipes*, *Pleurotus florida,* and *Volvariella volvacea* were investigated against human pathogenic bacteria. The antimicrobial activities of these mushrooms were evaluated following the agar well diffusion method against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and gram negative bacteria *Shigella dysenteriae*, *Salmonella typhi* and *Escherichia coli*. All the studied mushrooms displayed considerable antibacterial activity against all the pathogenic bacteria under study and it was found to be extraction solvent dependent.

Keywords: Mushrooms, antibacterial, minimum inhibitory concentration, zone of inhibition

The mushrooms are the fruiting bodies of macro fungi (Ascomycota and Basidiomycota) naturally occurring or cultivated, edible or non edible, either above ground or underground and large enough to be handpicked with unaided eyes (Chang and Miles, 1992). Mushrooms have medicinal properties such as antifungal, antibacterial, antiviral, antidiabetic, anticancer, immunomodulation, hepatoprotective, hypolipedimic and hyposensitive activities (Yang and Jong, 1989; Wasser and Weis, 1999; Ooi and Liu, 1999; Wasser, 2002; Jonathan and Fasidi, 2003 and Okhuoya *et al*., 2010).

In the present study, acetone, aqueous and methanol extracts of cultivated mushrooms viz, *Agaricus bisporus*, *Calocybe indica*, *F. velutipes*, *P. florida,* and *Volvariella volvacea* were tested for antibacterial properties against human pathogenic bacteria.

MATERIALS AND METHODS

Macrofungi and test organisms

The fruiting bodies of edible mushrooms *A. bisporus* (strain NBS-5), *C. indica* (DMRO-302), *F. velutipes* (DMRO-253), *P. florida* (DMRP-136) and *V. volvacea* (DMRO-484) were obtained in 2020-21 from ICAR-Directorate of Mushroom Research Solan, Himachal Pradesh, India. The test organism Gram positive bacteria *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and Gram negative bacteria *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli* were obtained from Department of Microbiology & Biotechnology, H.P. University, Summer Hill Shimla and IGMC, Shimla.

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Preparation of mushroom extracts

The fruiting bodies of *A. bisporus* (white button mushroom), *Volvariella volvacea* (paddy straw mushroom) *P. florida* (oyster mushroom), *C. indica* (milky mushroom) and *F. velutipes* (enoki, winter mushroom) were dried at 40-45°C in mushroom dryer under aseptic conditions. Dried fruiting bodies were powdered in a mixer grinder. Ten gram each of the powdered samples was soaked separately in 100 ml methanol, distilled water and acetone in an Erlenmayer flask. The flasks were covered with aluminum foil and kept at 29°C for seven days for extraction. After extraction, the samples were filtered through Whatman filter paper no. 1 and filterates were evaporated and dried at room temperature in case of methanol and acetone (Jonathan and Fasidi, 2003). Aqueous extracts were dried at 50°C in an oven. The extracts were collected and stored at temperature below 4°C in a refrigerator. Stock solution of 50 mg/ml of extracts was prepared by dissolving in dimethyl sulphoxide (DMSO) and water during antibacterial screening experiments.

Preparation of microbial inoculums

The young bacterial cultures were raised and used during the experiment. The nutrient broth media (Beef extract 1g, Peptone 5g, Sodium Chloride 1g, Yeast extract 2g, Distilled Water 1000 ml) was prepared and poured into several culture tubes. Then these culture tubes were sterilized in an autoclave, cooled and inoculated with the pure culture of bacterial strains maintained in the laboratory. The culture tubes were incubated in a BOD incubator at 37°C for 24 hrs compared against 0.4-0.5 McFarland turbidity standard tubes and was further used for the antibacterial assay.

Antibacterial Assay

The antibacterial activity of acetone, aqueous and methanol extract of five cultivated mushrooms was screened through agar well diffusion method. Throughout the investigations nutrient agar medium (Agar 20g, Peptone 5g, Yeast extract 2g, Beef extract 1g, Sodium Chloride 1g, Distilled Water 1000 ml) was used for the growth of pathogenic bacteria. The sterilization of medium was performed in an autoclave at 121.6°C for 30 minutes. The nutrient medium was poured in Petri plates under laminar flow hood and these plates were kept overnight at room temperature to check for any microbial contamination to appear. A 100µl of bacterial suspension from bacterial culture grown in nutrient broth for 24 hours was spread on surface of each nutrient agar plates. Five wells (one at centre and four at periphery of nutrient agar plate) of 8 mm diameter were carved with the help of sterilized steel cork borer in each Petri plate. The central wells in each plate were loaded with control (DMSO/Distilled water) and other wells with 25%, 50%, 75% and 100% concentration of prepared extracts of mushrooms. Streptomycin sulphate (10 ìg /ml) was used as positive control in a separate nutrient agar plate. These culture plates were incubated undisturbed at 37 ± 2°C for 24 hours in an incubation chamber. A zone of growth inhibition was observed around many loaded wells. The dimension of zone of inhibition was calculated by measuring the diameter of the inhibition zone (in mm) including the well diameter. The three measurements were taken in perpendicular direction in all three replicates of experiment and the average values were tabulated (Hemashenpagam and Selvaraj, 2010).

Determination of minimum inhibitory concentration (MIC)

The susceptibility of microorganisms to antimicrobial substances was determined by minimum inhibitory concentration (MIC) method. The MIC of mushroom extracts was determined through Resazurin dye reduction by using micro broth dilution assay. In cytotoxicity assays the Resazurin dye is used as an oxidation-reduction indicator for the evaluation of cell growth, (Mcnicholl *et al*., 2006). It is purple non fluorescent and nontoxic dye which becomes pink and fluorescent when reduced to resorufin in presence of

viable cells. The resorufin is further reduced to colourless hydroresorufin. The homogenous solution of Resazurin dye was made by dissolving 300 mg in 40 ml of sterile water. The microtiter plates (Tarson) having 96 micro wells were used for Resazurin dye based Microtiter Dilution Assay. The first well of every row in microtiter plate was loaded with 200 µl of mushroom extract (5mg/100µl). All other wells of row in microtiter plate were loaded with 100 µl of nutrient broth. Then transfer 100 µl of mushroom extract from first to subsequent wells in the row was performed to achieve two-fold serial dilution (throughout column), so that each well had 100 µl of mushroom extract in serially decreasing concentrations. Resazurin solution (10 µl) as an indicator was added in each well and finally, 10 µl of bacterial suspension was added to each well in the microtiter plate. In all microtiter plates one set each of positive control (antibiotic) and negative control (without mushroom extract) were also maintained. Each plate was loosely wrapped with cling film to avoid the dehydration of bacterial cultures. Plates were incubated in temperature-controlled incubator at 37±2°C for 24 hours and then observed for visual colour change in each well. The change in colour observed from purple to pink or colourless was taken as positive value, where bacterial culture was not inhibited. The value of the lowest concentration of mushroom extract at which colour changes as occurred in a particular test was taken as minimum inhibitory concentration.

The inhibition zone diameter (mm± SE) was calculated by measuring the inhibition zone in mutually perpendicular directions and taking the mean of the values in all three replicates.

RESULTS AND DISCUSSION

Acetone, aqueous and methanol extracts of all mushrooms (*A. bisporus*, *C. indica*, *F. velutipes*, *P. florida,* and *V. volvacea*) showed good antibacterial activity against all tested human pathogenic bacteria viz., *B. cereus*, *S. aureus*, *L. monocytogenes* and gram negative bacteria *S. dysenteriae*, *S. typhi*, *E. coli*.

The control did not show inhibition against the test bacteria. There was a gradual increase in inhibition zone diameter with increase in extract concentration (Table 1-3). All the extracts revealed different degree of antibacterial activity at a concentration of 100% (50 mg/ml) against the test bacteria. Antibacterial activity of mushrooms was extraction solvent dependent. The results of minimum inhibitory concentration (MIC) are shown in Table 4. The maximum antibacterial activity of acetone extracts was detected in *F. velutipes* against *E. coli* (16.17 ± 0.27 mm), aqueous extract *in P. florida* against *E. coli* (13.84 ± 0.61 mm) and methanol extract in *V. volvacea* against *L. monocytogenes* (15.21 ± 0.21 mm), while minimum activity of acetone extracts was detected in *P. florida* against *S. aureus* (12.24 ± 0.06 mm), aqueous extract in *C. indica* against *S. aureus* (10.63 ± 0.19) and methanol extract in *C. indica* against *S. aureus* (11.96 ± 0.28 mm). Results of the study showed that each cultivated mushroom has enough antibacterial property against both gram-positive and gram-negative bacteria.

In acetone extract of *A. bisporus* at 100% concentration the maximum zone of inhibition was against *E. coli* (14.80 ± 0.56 mm) followed by *L. monocytogenes*, *B. cereus*, *S. typhi*, *S. aureus* and *S. dysenteriae*. The zone of inhibition produced by aqueous extract of *A. bisporus* at 100% concentration was the minimum for all the pathogens tested. *A. bisporus* in methanol extract expressed maximum inhibition zone at 100% concentration against *B. cereus* (14.93 ± 0.28 mm) followed by *L. monocytogenes* , *S. typhi*, *S. dysenteriae*, *E. coli* and *S. aureus* (Table 1-3). The minimum inhibitory concentration (MIC) of acetone extract of *A. bisporus* was 0.625 mg/100µl against *B. cereus* and *E. coli* while 0.3125 mg/100µl against *S. aureus*, *S. dysenteriae*, *S. typhi* and *L. monocytogenes* . The

Table 1. Zones of inhibition shown by acetone extract of different cultivated mushrooms at different concentrations

Mushroom Extract	Conc. in %	Inhibition zone diameter (mm ± S.E.)					
		Bacillus cereus	***Staphylococcus aureus***	***Shigella dysenteriae***	***Salmonella typhi***	***Listeria. monocytogenes***	***Escherichia coli***
A. bisporus	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	12.22 ± 0.53	9.51 ± 0.16	10.20 ± 0.15	10.7 ± 0.29	12.00 ± 0.65	11.26 ± 0.30
	50	12.73 ± 0.50	10.10 ± 0.10	10.57 ± 0.13	11.00 ± 0.22	13.28 ± 0.51	12.48 ± 0.14
	75	13.46 ± 0.37	11.76 ± 0.18	11.43 ± 0.18	11.59 ± 0.29	13.49 ± 0.52	13.41± 0.28
	100	14.39 ± 0.62	12.46 ±0.12	12.26 ± 0.30	12.60 ± 0.19	14.51 ± 0.50	14.80 ± 0.56
C. indica	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	12.61 ± 0.31	11.33 ± 0.17	11.52 ±0.54	11.37 ± 0.21	12.50 ± 0.42	11.91 ±0.23
	50	12.74 ± 0.34	11.97 ± 0.36	12.44 ± 0.61	12.11 ± 0.35	13.12 ± 0.51	12.33 ± 0.26
	75	13.88 ± 0.38	12.51 ± 0.46	14.01 ± 0.27	12.82 ± 0.28	13.61 ± 0.39	13.30 ± 0.22
	100	15.01 ± 0.59	12.84 ± 0.48	15.31 ± 0.30	14.21 ± 0.29	14.48 ± 0.46	14.18 ± 0.36
F. velutipes	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	13.49 ± 0.23	11.98 ± 0.32	12.12 ± 0.28	12.72 ± 0.23	12.44 ± 0.46	12.69 ± 0.27
	50	14.13 ± 0.35	12.90 ± 0.33	12.61 ± 0.34	13.63 ± 0.21	13.44 ± 0.44	13.29 ± 0.36
	75	14.92 ± 0.44	13.50 ± 0.35	13.34 ± 0.38	14.12 ± 0.29	14.60 ± 0.65	13.87 ± 0.43
	100	15.80 ± 0.71	13.83 ± 0.39	13.87 ± 0.47	14.99 ± 0.51	15.64 ± 0.59	16.17 ± 0.27
P. florida	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	12.47 ± 0.55	10.88 ± 0.16	11.76 ±0.43	11.33 ± 0.38	10.74 ± 0.32	11.37 ± 0.23
	50	13.13 ± 0.64	11.27 ± 0.11	12.30 ± 0.46	12.50 ± 0.58	11.81 ± 0.27	12.68 ± 0.40
	75	13.86 ± 0.78	11.67 ± 0.07	13.51 ± 0.42	13.43 ± 0.58	12.77 ± 0.46	13.21 ± 0.34
	100	14.80 ± 0.80	12.24 ± 0.06	14.12 ± 0.51	14.16 ± 0.54	13.80 ± 0.34	13.87 ± 0.41
V. volvacea	Control	00±00	00±00	00±00	00±00	00±00	00±00
	25	12.08 ± 0.55	11.43 ± 0.38	11.01 ± 0.28	10.07 ± 0.27	12.42 ± 0.29	11.43 ± 0.14
	50	12.92 ± 0.69	12.34 ± 0.45	12.60 ± 0.34	10.88 ± 0.37	13.12 ± 0.32	11.78 ± 0.20
	75	13.50 ± 0.72	13.27 ± 0.41	13.89 ± 0.47	11.91 ± 0.40	13.91 ± 0.50	12.33 ± 0.32
	100	14.30 ± 0.84	13.85 ± 0.62	15.84 ± 0.61	12.34 ± 0.49	14.47 ± 0.50	13.06 ± 0.35

mm: Milli meter, S.E.: Standard error

MIC of aqueous extract of *A. bisporus* was 0.625 mg/100µl against *L. monocytogenes* and 1.25 mg/ 100µl against *B. cereus, E. coli S. aureus*, *S. dysenteriae* and *S. typhi*. The MIC of methanol extract of *A. bisporus* was 1.25 mg/100µl against *S. typhi* and 0.625 mg/100µl against *B. cereus, E. coli S. aureus*, *S. dysenteriae* and *L. monocytogenes* (Table 4).

Similar antibacterial activity of methanol extract of *A. bisporus* was reported by other studies comparable to our results. Chellal and Lukasova (1995) documented antibacterial activity of *A. bisporus* against *B. subtilis* (12 mm) and *S. aureus* (18-22 mm). Jagadish *et al*. (2009) documented antibacterial activity of ethanol extract of *A. bisporus* against *S. aureus* (18-22 mm) and *B. subtilis* (12 mm) but no growth inhibition against *E. coli*. Akyüz *et al*. (2010) reported antibacterial activity of *A. bisporus* against *Bacillus sp., S. aureus, E. coli,* by disk diffusion method. However Priya and Srinivasan, (2013) did not detect any zone of growth inhibition against *Bacillus sp., S. aureus, E. coli* by disk diffusion method and stated that these bacterial species were resistant to *A. bisporus*. Padmavathy *et al*. (2014) reported 15 mm zone of growth inhibition in petroleum ether extract of *A. bisporus* against *S. aureus*. Antibacterial activity of acetone and methanol extracts of fruiting bodies of *A. bisporus* from DMR Solan was also reported by Sharma *et al*. (2015) which was comparable with present study. Ndungutse *et al*. (2015) reported antibacterial activity of *A.*

bisporus by using acetone, ethanol and water extract against *E. coli* and *S. aureus.* Hussein *et al*. (2018) reported antibacterial activity of ethanol extract against *S. aureus* (15 mm) and *S. typhi* (9 mm).

Acetone extract of *C. indica* at 100% concentration showed maximum inhibition zone in *S. dysenteriae* (15.31 ± 0.30 mm) followed by *B. cereus*, *L. monocytogenes* , *S. typhi*, *E. coli* and *S. aureus*. In case of aqueous extract of *C. indica*, the zone of inhibition was observed to be the maximum against *E. coli* (12.62 ± 0.47 mm) followed by *L. monocytogenes*, *S. typhi*, *S. dysenteriae*, *B. cereus* and *S. aureus*. Methanol extract of *C. indica* at 100% concentration showed maximum inhibition zone against *B. cereus* (14.01 ± 0.52 mm) followed by *S. typhi*, *E. coli*, *L. monocytogenes* , *S. dysenteriae* and *S. aureus* (Table 1-3). The MIC of acetone extract of *C. indica* extract was 0.625 mg/100µl against *B. cereus,* and 0.3125 mg/100µl against all other test bacteria. The MIC in aqueous extract of *C. indica* was 2.5 mg/100µl against *B. cereus* and 1.25 mg/100µl against rest of the test bacteria. The MIC of methanol extract of *C. indica* was 1.25 mg/100µl against *B. cereus, S. typhi* and 0.625 mg/100µl against *S. aureus*, *S. dysenteriae*, *L. monocytogenes* and *E. coli* (Table 4).

Giri *et al*. (2012) have detected bactericidal activity of methanol extract of *C. indica* against *E. coli* but did not detect against *B. cereus* and *S. aureus*. Padmavathy *et al*. (2014) reported

Table 2. Zones of inhibition shown by aqueous extract of different cultivated mushrooms at different concentrations

Mushroom Extract	Conc. in %	Inhibition zone diameter (mm ± S.E.)					
		Bacillus cereus	***Staphylococcus aureus***	***Shigella dysenteriae***	***Salmonella typhi***	***Listeria. monocytogenes***	***Escherichia coli***
A. bisporus	Control	00±00	00±00	00±00	00±00	00±00	00±00
	25	8.87 ± 0.21	9.17 ±0.14	8.88 ± 0.16	9.43 ± 0.08	9.31 ± 0.21	9.07 ± 0.26
	50	9.71 ± 0.14	9.70 ± 0.15	9.54 ± 0.16	9.89 ± 0.05	9.83 ± 0.21	10.09 ± 0.05
	75	10.33 ± 0.18	10.34 ± 0.15	10.22 ± 0.13	10.31 ± 0.15	10.24 ± 0.16	10.16 ± 0.24
	100	11.20 ± 0.09	10.76 ± 0.25	10.91 ± 0.21	10.99 ± 0.17	11.17 ± 0.35	11.23 ± 0.16
C. indica	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	8.57 ± 0.14	9.06 ± 0.04	9.04 ± 0.04	9.76± 0.18	9.33 ± 0.15	9.51 ± 0.12
	50	8.68 ± 0.16	9.57 ± 0.02	9.62 ± 0.03	10.61 ± 0.13	9.92 ± 0.07	10.43 ± 0.25
	75	9.89 ± 0.18	9.96 ± 0.06	10.24 ± 0.10	11.07 ± 0.09	10.81 ± 0.10	11.44 ± 0.39
	100	10.69 ± 0.27	10.63 ± 0.19	10.83 ± 0.07	11.73 ± 0.11	12.02 ± 0.25	12.62 ± 0.47
F. velutipes	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	9.62 ± 0.49	10.03 ± 0.45	10.33 ± 0.37	10.48 ± 0.32	9.72 ± 0.23	9.66 ± 0.31
	50	10.39 ± 0.53	10.66 ± 0.49	11.27 ± 0.65	11.66 ± 0.58	10.71 ± 0.35	11.03 ± 0.50
	75	11.33 ± 0.41	11.62 ± 0.48	12.28 ± 0.91	12.77 ± 0.77	11.68 ± 0.36	12.21 ± 0.71
	100	12.50 ± 0.64	13.26 ± 0.48	13.49 ± 0.86	13.79 ± 0.81	12.92 ± 0.31	13.84 ± 0.61
P. florida	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	8.76 ± 0.05	8.79 ± 0.19	9.13 ± 0.21	9.48 ± 0.03	9.51 ± 0.06	9.43 ± 0.21
	50	9.27 ± 0.09	9.54 ± 0.16	9.67 ± 0.18	9.86 ± 0.06	10.29 ± 0.07	10.06 ± 0.29
	75	10.44 ±0.06	10.19 ± 0.15	10.84 ± 0.08	10.44 ± 0.05	10.61 ± 0.07	10.78 ± 0.32
	100	11.88 ± 0.07	11.27 ± 0.23	11.97 ± 0.09	11.14 ± 0.13	11.40 ± 0.15	11.91 ± 0.51
V. volvacea	Control	00±00	00±00	00±00	00±00	00±00	00±00
	25	9.13 ± 0.18	9.47 ± 0.26	9.66 ± 0.09	8.78 ± 0.20	8.82 ± 0.20	9.21 ± 0.16
	50	9.57 ± 0.14	10.11 ± 0.17	10.00 ± 0.08	9.38 ± 0.25	9.43 ± 0.19	9.89 ± 0.09
	75	10.80 ± 0.27	10.58 ± 0.20	10.69 ± 0.09	10.46 ± 0.07	10.59 ± 0.35	10.83 ± 0.27
	100	12.31 ± 0.11	11.51 ± 0.24	12.08 ± 0.15	12.10 ± 0.10	12.02 ± 0.38	12.08 ± 0.25

mm: Milli meter, S.E.: Standard error

Table 3. Zones of inhibition shown by methanol extract of different cultivated mushrooms at different concentrations

Mushroom Extract	Conc. in %	Inhibition zone diameter (mm ± S.E.)					
		Bacillus cereus	*Staphylococcus aureus*	*Shigella dysenteriae*	*Salmonella typhi*	*Listeria. monocytogenes*	*Escherichia coli*
A. bisporus	Control	00±00	00±00	00±00	00±00	00±00	00±00
	25	10.61 ± 0.18	11.53 ± 0.50	11.29 ± 0.11	10.33 ± 0.08	10.63 ± 0.19	10.82 ± 0.10
	50	13.09 ± 0.26	11.71 ± 0.21	11.92 ± 0.14	11.64 ± 0.09	12.59 ± 0.24	11.44 ± 0.10
	75	14.07 ± 0.25	12.49 ± 0.41	13.13 ± 0.20	13.09 ± 0.15	13.83 ± 0.10	12.19 ± 0.18
	100	14.93 ± 0.28	13.06 ± 0.23	14.09 ± 0.26	14.34 ± 0.27	14.82 ± 0.12	13.49 ± 0.19
C. indica	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	11.11 ± 0.26	9.83 ± 0.44	10.18 ± 0.16	11.40 ± 0.27	10.89 ± 0.17	10.38 ± 0.19
	50	11.68 ± 0.37	10.81 ± 0.25	11.09 ± 0.18	11.74 ± 0.33	11.69 ± 0.22	11.19 ± 0.18
	75	12.56 ± 0.31	11.62 ± 0.26	12.18 ± 0.18	12.81 ± 0.38	12.38 ± 0.36	11.79 ± 0.34
	100	14.01 ± 0.52	11.96 ± 0.28	12.81 ± 0.24	13.78 ± 0.41	13.01 ± 0.45	13.26 ± 0.33
F. velutipes	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	11.27 ± 0.23	10.47 ± 0.16	9.91 ± 0.09	10.99 ± 0.28	10.66 ± 0.29	10.27 ± 0.08
	50	11.66 ± 0.24	10.98 ± 0.08	10.44 ± 0.11	11.56 ± 0.19	11.28 ± 0.40	10.70 ± 0.05
	75	12.34 ± 0.27	11.58 ± 0.04	11.08 ± 0.22	12.36 ± 0.20	11.71 ± 0.36	11.71 ± 0.25
	100	13.18 ± 0.45	12.11 ± 0.29	12.01 ± 0.27	13.41 ± 0.37	12.63 ± 0.36	12.49 ± 0.23
P. florida	Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	25	11.10 ± 0.09	9.19 ± 0.27	10.62 ± 0.19	10.59 ± 0.17	10.83 ± 0.17	10.89 ± 0.26
	50	11.63 ± 0.16	10.69 ± 0.56	11.54 ± 0.28	11.67 ± 0.08	11.23 ± 0.16	11.52 ± 0.26
	75	12.16 ± 0.29	12.52 ± 0.37	12.57 ± 0.50	12.70 ± 0.19	12.37 ± 0.19	12.44 ± 0.20
	100	13.32 ± 0.51	13.12 ± 0.33	13.74± 0.30	13.19 ± 0.13	13.43 ± 0.20	13.02 ± 0.26
V. volvacea	Control	00±00	00±00	00±00	00±00	00±00	00±00
	25	10.57 ± 0.14	10.07 ± 0.13	11.24 ± 0.30	11.94 ± 0.34	12.68 ± 0.14	10.83 ± 0.11
	50	11.52 ± 0.17	11.48 ± 0.13	11.77 ± 0.32	12.27 ± 0.36	13.14 ± 0.12	11.16 ± 0.16
	75	12.20 ± 0.30	12.34 ± 0.17	12.41 ± 0.36	12.79 ± 0.39	13.58 ± 0.16	12.14 ± 0.23
	100	13.70 ± 0.20	14.92 ± 0.13	13.73 ± 0.32	14.01 ± 0.32	15.21 ± 0.21	13.32 ± 0.06

mm: Milli meter, S.E.: Standard error

Table 4. Minimum Inhibitory Concentration (mg/100µl) of different extracts of cultivated mushroom against various pathogenic bacteria

Mushroom	Extract	*Bacillus cereus*	*Staphylococcus aureus*	*Shigella dysenteriae*	*Salmonella typhi*	*Listeria. monocytogenes*	*Escherichia coli*
A. bisporus	Acetone	0.625	0.3125	0.3125	0.3125	0.3125	0.625
	Aqueous	1.25	1.25	1.25	1.25	0.625	1.25
	Methanol	0.625	0.625	0.625	1.25	0.625	0.625
C. indica	Acetone	0.625	0.3125	0.3125	0.3125	0.3125	0.3125
	Aqueous	2.5	1.25	1.25	1.25	1.25	1.25
	Methanol	1.25	0.625	0.625	1.25	0.625	0.625
F. velutipes	Acetone	0.625	0.3125	0.3125	0.3125	0.3125	0.3125
	Aqueous	1.25	1.25	1.25	1.25	0.625	1.25
	Methanol	1.25	0.625	0.625	1.25	0.625	0.625
P. florida	Acetone	0.625	0.3125	0.3125	0.625	0.3125	0.3125
	Aqueous	1.25	1.25	1.25	1.25	1.25	1.25
	Methanol	1.25	0.625	0.625	1.25	0.625	0.625
V. volvacea	Acetone	0.625	0.3125	0.625	0.625	0.3125	0.3125
	Aqueous	0.625	1.25	0.625	1.25	1.25	1.25
	Methanol	0.625	0.3125	0.625	0.625	0.625	0.625

antibacterial activity in petroleum ether extract of *C. indica* against *S. aureus* (10 mm) and Krishnaveni and Manikandan (2014) observed bactericidal properties of *C. indica* against *E. coli* and *Klebsiella pneumoniae*. Datta *et al*. (2020) checked antibacterial activity and minimum inhibitory concentration (MIC) of different extracts from *C. indica* against pathogenic bacteria such as *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, S. aureus*, and *S. epidermidis*, using the broth dilution and agar well diffusion method.

F. velutipes in acetone extract showed maximum zone of inhibition against *E. coli* (16.17 ± 0.27 mm) followed by *B. cereus*, *L. monocytogenes* , *S. typhi*, *S. dysenteriae* and *S. aureus*. In aqueous extract the maximum inhibition zone against *E. coli* (13.84 ± 0.61mm) followed by *S. typhi, S. dysenteriae*, *S. aureus*, *Listeria monocytogenes* and *B. cereus*. The methanol extract showed the maximum inhibition zone against *S. typhi* (13.41 ± 0.37 mm) followed by *B. cereus*, *L. monocytogenes* , *E. coli*, *S. aureus* and *S. dysenteriae* (Table 1-3). The MIC of acetone extract of *F. velutipes* was 0.625 mg/100µl against *B. cereus* and 0.3125 mg/100µl against all other test bacteria. The MIC of aqueous extract of *F. velutipes* was 0.625 mg/100µl against *L. monocytogenes* and 1.25 mg/100µl against rest of the pathogens tested. The MIC of methanol extract of *F. velutipes* was 1.25 mg/100µl against *B. cereus*, *S. typhi* and 0.625 mg/100µl against *S. aureus*, *L. monocytogenes, S. dysenteriae*, and *E. coli* (Table 4).

Karaman *et al*. (2010) showed that both methanol and chloroform extracts from mature fruiting bodies of *F. velutipes* exhibited strong antibacterial activities, particularly against *S. aureus* and *Bacillus subtilis*. The similar studies on antibacterial activity of methanol extract of Macedonian wild *F. velutipes* was conducted against both Gram-positive and Gram-negative bacteria, including *B. subtilis, Bacillus pumilus, S.aureus,* and *Pseudomonas aeruginosa* (Nedelkoska et al., 2013). Nwe and Zin (2020) reported that ethanol extract of *F. velutipes* had more antimicrobial activity against *Bacillus subtilis* (14.1 ± 0.2 mm), *E. coli* (11.5 ± 0.1 mm), *S. typhi* (15.5± 0.1 mm) and *S. aureus* (13.5 ± 0.1) than aqueous extract *Bacillus subtilis* (9.0± 0.2 mm), *E. coli* (11.0 ± 0.1mm), *S. typhi* (7.0± 0.1 mm) *and S. aureus* (no activity). Shah *et al*. (2018) had stated that aqueous extract of *F. velutipes* exhibited higher antibacterial activity than methanol and acetone extract.

P. florida in acetone extract had shown maximum inhibition zone against *B. cereus* (14.80 ± 0.80 mm) followed by *S. typhi*, *S. dysenteriae*, *E. coli*, *L. monocytogenes* and *S. aureus*. In aqueous extract *P. florida* showed maximum inhibition zone against *S. dysenteriae* (11.97 ± 0.09 mm) followed by *E. coli*, *B. cereus*, *Listeria. monocytogenes*, *S. aureus* and *S. typhi*. The methanol extract of *P. florida* had shown the maximum inhibition zone against *S. dysenteriae* (13.74 ± 0.30 mm) followed by *L. monocytogenes* , *B. cereus*, *S. typhi*, *S. aureus* and *E. coli* (Table 1-3). The MIC of *P. florida* in acetone extract was 0.625 mg/100µl against *B. cereus, S. typhi* and 0.3125 mg/100µl against other test pathogens. The MIC of aqueous extract of *P. florida* was 1.25 mg/100µl against all test bacteria. The MIC of methanol extract was 1.25 mg/100µl against *B. cereus, S. typhi* and 0.625 mg/100µl against *S. aureus*, *S. dysenteriae*, *L. monocytogenes* and *E. coli* (Table 4).

Iwalokun *et al*. (2007) reported antimicrobial activity of acetone and petroleum ether extract of *Pleurotus ostreatus* against *Bacillus* sp. (7.1-7.8 mm), *Candida* sp. (8.0-8.3 mm), *and E. coli* (7.0-8.2 mm), *Klebsiella pneumoniae* (7.0-7.1 mm), *S. typhi* (7.0-7.5 mm) and *Staphylococcus* sp. (7.0-7.6mm). Jagadish *et al*. (2008) reported antimicrobial activity of *P. florida* in ethanol extract against *S. aureus* (16.0 mm) and *E. coli* (12.0 mm). Methanol extract of *P. florida* showed activity against *E.coli* (21 ± 0.9 mm), *S. typhi* (20 ± 0.5 mm), *S. aureus*

(20 ± 0.4 mm), *Camphylobacter sp.* (19 ± 0.8 mm), *Bacillus sp.* (14 ± 0.5 mm), *Pseudomonas sp.* (8 ± 0.5 mm), *Klebsiella sp.* (12 ± 0.6 mm) and *Vibrio sp.* (20 ± 0.9 mm) (Menaga *et al.* 2012). Antibacterial activity in methanol extract of *P. florida* against gram positive bacteria *S. aureus* reported by Muthukumaran *et al*. (2014) by agar well diffusion method was in agreement with present study. The results of our study regarding bactericidal activity of *P. florida* against *E. coli* were in accordance to Krishnaveni and Manikandan (2014).

Acetone extract of *V. volvacea* at 100% concentration showed maximum inhibition zone against *S. dysenteriae* (15.84 ± 0.61 mm) followed by *L. monocytogenes* , *B. cereus*, *S. aureus*, *E. coli* and *S. typhi*. Aqueous extract of *V. volvacea* showed maximum inhibition zone against *B. cereus* (12.31 ± 0.11 mm) followed by *S. typhi*, *E. coli*, *S. dysenteriae*, *L. monocytogenes* and *S. aureus*. Methanol extract of *V. volvacea* showed maximum inhibition zone against *L. monocytogenes* (15.21 ± 0.21 mm) followed by *S. aureus*, *S. typhi*, *S. dysenteriae*, *B. cereus* and *E. coli* (Table 1-3). The MIC of acetone extract of *V. volvacea* was 0.625 mg/100µl against *B. cereus, S. typhi, S. dysenteriae* and 0.3125 mg/100µl against other test pathogens. The MIC of aqueous extract of *V. volvacea* was 0.625 mg/100µl against *B. cereus, S. dysenteriae* and 1.25 mg/100µl against others. The MIC of methanol extract of *V. volvacea* was 0.3125 mg/100µl against *S. aureus* and 0.625 mg/100µl against *B. cereus, S. dysenteriae, S. typhi, L. monocytogenes,* and *E. coli* (Table 4).

Results of present study are in agreement with previous reports. Perera *et al*. (2001) reported the high antibacterial activity of *V. volvacea* against *E. coli*. Ayodele and Idoko (2011) reported antibacterial activity of *V. volvacea* against *S. aureus* (10.4 ± 0.05 mm) and *E. coli* (7.7 ± 0.05 mm). Giri *et al*. (2012) reported that *V. volvacea* had antibacterial activity against *B. cereus* and *E. coli*, but had no activity against *S. aureus* which is contrary to our study. Methanol extract of *V. volvacea* had shown effective bactericidal properties at concentration of 1 mg/ml in DMSO against *E. coli, B. cereus, S. aureus, Klebsiella pneumoniae* and *Proteus vulgaris* (Ali *et al*., 2018).

CONCLUSION

In the present investigations, all the mushroom species under study (*A. bisporus*, *C. indica*, *F. velutipes*, *P. florida,* and *V. volvacea*) displayed a considerable antibacterial potential against all the human pathogens (*B. cereus*, *S. aureus*, *L. monocytogenes S. dysenteriae*, *S. typhi* and *E. coli*). So, there is a need for further studies to isolate and characterize the bioactive compounds present in cultivated mushrooms and these metabolites can be used to develop effective drugs against these human pathogenic bacterial strains. Cultivated mushrooms due to their high nutritional values and good antibacterial properties strongly support the saying of Hippocrates “let the food be your medicine and medicine be your food”.

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