Indian Journal of Natural Products and Resources
Vol. 8(3), September 2017, pp.254-262

Antimicrobial activity and bioactive compounds of Indian wild Mushrooms

Hirut Tsegaye Feleke* and Anila Doshi
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur-313001, India

Received 04 April 2016; Revised 29 June 2017

Ethanol extracts from the fruiting bodies of three Indian wild mushrooms; *Leucopaxillus giganteus*, *Phellorinia inquinans*, and *Podaxis pistillaris* were investigated for their antimicrobial activities by filter paper disc and well diffusion methods. The extracts inhibited the growth of plant pathogenic bacteria such as *Xanthomonas axonopodis* pv. *punicae*, *Bacillus pumilus*, and *Pseudomonas syringe;* and plant pathogenic fungi like *Aspergillus niger, A. solani*um*, Alternaria solani*, *Colletotrichum graminicola, Fusarium oxysporum, F. solani*, and *Helminthosporium maydis.* Well diffusion method gave a better inhibition zone, and the plant pathogenic bacteria were found to be more sensitive to the extracts than the fungi. The minimum inhibition concentration was the least for *L. giganteus* than *P. inquinans* and *P. pistillaris* against the respective plant pathogenic bacteria. In general, ethanol extracts from the fruiting bodies of *L. giganteus* were found to be superior in antimicrobial activities over the other species investigated. The bioactive compounds in this wild edible mushroom also determined.

Keywords: Antimicrobial activities, Bioactive compounds, Extraction, Inhibition, Wild mushrooms.

IPC code; Int. cl. (2015.01)−A23L 1/28, A61K 36Q/00

Introduction

In addition to serving as a source of food, wild mushrooms are reported to have antimicrobial activities: inhibitory effect on growth and germination of *Alternaria alternate*[1]; antimicrobial activities on *Botrytis cinera, Colletotrichum gloeosporioides*, and *C. miyabeanus*[2] and antagonistic effects of *Leucopaxillus tricolor* on *Aspergillus fumigatus* and *Fusarium dimerium*[3]. The anti-feeding effects of some mushroom strains like *Lactarius vellereus* and *L. rufus* against some insects were also documented in the literature[4]. The reports revealed the potential and practical applicability of wild mushrooms for controlling plant pathogens.

It is known that macrofungi need antibacterial and antifungal compounds to survive in their natural environment. These compounds can be isolated from many mushroom species and may be used for human benefits. Such bioactive molecules reported to be exist in both edible and non edible mushroom species[5]. Bioactivities of mushrooms include antibacterial, antifungal, antioxidant, antiviral, anti-tumor, cytostatic, immunosuppressive, antiallergic, antiatherogenic, hypoglycemic, anti-inflammatory, and hepatoprotective activities[6]. The responsible bioactive compounds belong to several chemical groups which are often polysaccharides or triterpenes. One macrofungi species can have various bioactive compounds and pharmacological effects[6].

A few studies have been reported on the antimicrobial activity of Indian wild edible mushrooms. *Leucopaxillus giganteus* (Sowerby) is a wild mushroom which belongs to phylum Basidiomycete, order Agaricales, and family Tricholomataceae[7]. The *L. giganteus* used in the present study were collected from Rajasthan (India) with detailed morphological, anatomical, and physiological characteristics study was carried out by Doshi in 2013 while working with All Indian Coordinated Mushroom Improvement Project Solan, Himachal Pradesh, India. *Phellorinia inquinans* and *Podaxis pistillaris* are also known as wild edible mushrooms and are used as a traditional medicine in some parts of the world[8]. These mushrooms were reported to be found in India[9,10]. To the best of the author's knowledge, report about the antimicrobial and bioactive compounds of these species in India is not available. Therefore, the aim of the present study was to evaluate the antimicrobial activity of *L. giganteus, P. Inquinansi*, and *P. pistillaris* against selected plant

*Correspondent author
E-mail:hirazershye@gmail.com, segayehirut@yahoo.com
Tel.: 0294-2417835
Fax: 0294-2418976

pathogens and to determine the major bioactive compounds present in the ethanol extracts of these species.

Materials and Methods

Experimental materials

The fruiting bodies of *L. giganteus, P. Inquinans*, and *P. pistillaris* were obtained from All Indian Coordinated Mushroom Improvement Project (AICRP), Department of Plant Pathology, Rajasthan College of Agriculture (RCA), Udaipur, India. These species were collected from different forest areas of Rajasthan (Northwest of India) in the rainy season of 2013. The basidiomycetes were identified by their spore prints and comparing their morphological, anatomical, and physiological characteristics with the standard descriptions[11,12]. Except *Xanthomonas axonopodis pv. Punicae and Helminthosporium maydis* which were obtained from Department of Plant Pathology, RCA, all test cultures were procured from Microbial Type Culture Collection and Gene Bank (MTCC) of CSIR-Institute of Microbial Technology, Chandigarh, India. The procured bacterial cultures were *Bacillus pumillus* (MTCC 297) and *Pseudomonas syringae* (MTCC 1604), while the fungi were *Aspergillus solani* (MTCC 377*), Fusarium solani* (2671), *F. oxysporium* (MTCC 284), *Alternaria solani* (MTCC 2101), *Colletotrichum graminicola* (MTCC 3405) and *A. niger* (MTCC 404).

Preparation of crude ethanol extracts

The fruit bodies of each of the mushroom species were cut into small pieces and oven dried at 40 ^{o}C. The dried carpophore was pulverized in a Moulinex blender. Each powder carpophore (10 g) was soaked separately in 100 mL of 95 % ethanol in an Erlenmeyer flask. The flask was covered with aluminium foil and kept at 25 ^{o}C for 7 days. After 7 days, the content of the flask was filtered with Whatman filter paper No. 1.

The filtrate obtained was concentrated in a rotary evaporator at 40 ^{o}C. The extraction was done in replicates of four and the average yield was reported. The dried extract was stored in a refrigerator at -4 ^{o}C for further analysis[13].

Antimicrobial activity test of the crude extracts

Antibacterial activity

Antibacterial activities of the ethanolic extract of the different mushrooms were determined by Disc and Well methods[14]. *B. pumilus* was inoculated in trypticase (tryptic) soy broth (15 % tryptone, 5 % soybean meal, and 5 % NaCl), while *X. axonopodis* pv. *punicae* and *P. syringae* were cultured in nutrient broth (5 % peptone, 5 % NaCl, 1.5 % beef extract, and 1.5 % yeast) and growth media 3 (GM 3) broth (0.5 % peptone, 0.5 % sodium chloride, 0.1 % beef extract, and 0.2 % yeast extract), respectively. The Inoculants were incubated at 27±1 °C for 24 h. The culture suspensions were then prepared and adjusted by comparing against 0.4-0.5 Mc Farland turbidity standard tubes. Trypticase soy agar (TSA), Nutrient agar and GM 3 agar (20 mL each) were prepared and poured into different sterilized petri dish (10 × 90 mm diameter) after injecting 100 µL of the respective bacterial culture suspension. The media were distributed in Petri dishes homogenously.

The ethanolic extracts obtained from the dried mushrooms were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 mg/mL and sterilized by filtration through a 0.22 µm membrane filter[15,16]. Sterile filter paper discs of 5 mm diameter were soaked for 10 sec in the extracts and dried at 40 °C for 1 h. The paper disc then placed over the surface of the medium, and incubated at 27 °C for 24 h. Similarly, in the case of well method, a well of 6 mm in diameter was bored at the centre of each solidified media with the help of sterilized cork borer. The mushroom extracts (100 µL) were filled into the wells directly. At the end of the incubation period, the inhibition zone (diameter) formed on each media were measured. Inhibitory activity of the DMSO was also checked as negative control. Studies were performed in four replications and the results were expressed as average values. Standard antibiotics, tetracycline (30 µg) and penicillin (10 µg) were used as the reference discs.

Antifungal activity

The plant pathogenic fungal pure cultures (*A. solani, A. solanium*, *A. niger, C. graminicola, F. solani, F. oxysporum*, and *H. maydis)* were incubated at room temperature for 48 h in liquid Potato Dextrose media. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 Mc Farland turbidity standard tubes. The Potato Dextrose Agar (PDA) media (20 mL) was poured into sterilized petri dishes (10 x 90 mm diameter) after inoculation with the fungal cultures (100 µL) and distributed homogeneously, and allowed to solidify. With the help of sterilized cork borer, a

well of 6 mm in diameter was bored at the centre of the media in the plate. The mushrooms extracts of 100 µL was filled into the wells of agar plates directly. The plates were incubated at 28±1 °C for 4 days. Grisofulvin (100 µg/mL) were used as reference. After the incubation period, the zone of inhibition was measured, tabulated and compared with the reference.

Determination of minimum inhibitory concentration (MIC)

The standard agar dilution protocol with double dilution was used to determine MIC. The MICs of the extract for each test microorganism were regarded as the agar plate with the lowest concentrations without growth[17,18]. The standard concentration of the extract used was 20.0 mg/mL. DMSO was used as diluent. Mushrooms with activities at this concentration were regarded as having antimicrobial properties while others with no activity at this concentration were disregarded[19]. The MIC determination was done in 4 replications and the average MICs were reported.

Determination of bioactive components

Total phenols

Phenolics compounds in the mushroom extracts were determined by colorimetric assay with some modifications[20]. Briefly, 1 mL of sample was mixed with 1 mL of Folin-Ciocalteu phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to calculate the standard curve (0.01-0.4 mM). The results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Total flavonoid concentration

Flavonoid contents in the mushroom extracts were measured by a colorimetric method[21]. The extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a 5 % $NaNO_2$ solution. After 5 min, 150 µL of a 10 % $AlCl_3$+H_2O solution was added. After another 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed well and the intensity of pink colour was measured at 510 nm. (+)-chatechin was used to calculate the standard curve (0.022-0.34 mM). The results were expressed as mg of (+)-chatechin equivalents (CEs) per g of extract.

Concentration of β-carotene and lycopene

The concentration of *β*-carotene and lycopene in the mushroom extracts were determined following appropriate method[22]. The dried ethanolic extract (100 mg) was vigorously shaken 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. Content of *β*-carotene and lycopene concentrations were calculated as follows:

$$Lycopene\ (mg/\ 100\ mL) = -0.0458\ A663 + 0.372\ A505 —.0806\ A453 \quad \ldots (Eq.\ 1)$$

$$\beta - carotene\ (mg/\ 100\ mL) = 0.216\ A663 - 0.304\ A505 + 0.452\ A453\ldots \quad \ldots (Eq.\ 2)$$

The results were expressed as mg of carotenoid per g of extract.

Concentration of ascorbic acid

Ascorbic acid concentrations in the mushrooms extract were estimated as given in the literature[23]. The dried ethanolic extract (100 mg) was extracted with 10 mL of 1 % metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2,6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of L-ascorbic acid (0.020-0.12 mg/mL) with 99 % purity. The results were expressed as mg of ascorbic acid/g of extract.

In all bioactive component determinations, the assays were carried 4 times and the results were mean±standard deviation.

Results and Discussion

Ethanolic extraction products of Indian wild mushrooms

The yields of ethanol extracts from fruiting bodies of dried mushrooms (10 g) were determined. The yield (g) and % extract (w/w) were 0.38 and 3.78 from *P. pistillaris,* 0.57 and 5.7 from *P. inquinans,* and 1.46 and 14.57 from *L. giganteus,* respectively. It was observed that *L. giganteus* gave the maximum amount of extracts followed by *P. inquinans* and *P. pistillaris*. The result implied that most of the soluble components in mushrooms were high in polarity. There was significant difference (p <0.05) in the extraction yield between the three wild mushrooms used. The findings were comparable with the previous report[24]. Discrepancy in the extraction yield from fruiting bodies of the mushrooms might occur due to the difference in strains and harvest times[25].

Antimicrobial activity

The antimicrobial effect of ethanol extracts of the three Indian wild mushrooms; *P. pistillaris, P. inquinans* and *L. giganteus* were checked against one Gram-positive and two Gram-negative species of bacteria, and seven species of plant pathogenic fungi. All the mushrooms used in the present study exhibited various degrees of antimicrobial properties against the plant pathogens checked. The antimicrobial activities were confirmed by the appearance of clear zone around the mushroom extracts that inhibit the growth of those pathogens on the media.

Antibacterial activities

Ethanol extracts of the mushrooms used in the present investigation possessed varying degrees of antibacterial properties against the bacteria tested (Table 1). *L. giganteus* produced the largest zone of inhibition (45.83 and 44.17 mm) against *B. pumilus* and *P. syringe*, respectively, followed by *X. axonopodis pv. punicae* (35 mm). The strong antibacterial properties possessed by *L. giganteus* might be associated with the presence of medicinal ingredients in the fungus (Clitocinet) which can be used to treatment cervical, ovarian, and endometrial cancers[26]. The potent antibacterial activity exhibited by *L. giganteus* against most of the tested bacteria supports the previous report[27].

According to the present findings, *P. inquinans* exhibited inhibitory effect against all tested bacterial strains. The highest antibacterial activities of *P. inquinans* were 42.67 and 31.33 mm against *P. syringe* and *B. pumilus*, respectively. These results were in accordance with earlier studies[28,29]. The *Phellinus igniarius* extracts showed highest antibacterial activity against *Bacillus pumilus, B. subtilis, Pseudomonas aeruginosa, Escherichia coli and Sarcina lutea*[24]. In a previous report, the fruiting body extract of *Phellorinia herculean* showed maximum inhibition zone against *P. aeruginosa* (25 mm) than other tested bacteria; *B. subtilis* (20 mm) and *Staphyloccocus aureus* (18 mm)[30].

P. pistillaris produced inhibitory zones of 40.33, 29.33, and 14.97 mm against *B. pumilus, P. Syringe*, and *X. axonopodis* pv. *punicae* by well method, respectively (Table 1). It is reported that with paper disc method, *P. pistillaris* exhibits a strong antibacterial activity against several Gram-positive and Gram-negative bacteria such as *Micrococcus flavus, B. subtilis, Proteus mirabilis and E. Coli*[30]. The present investigation revealed that well diffusion method was more effective to show the antimicrobial activities of the mushrooms extracts as compared to filter paper disc method. Plate 1 and 2 showed the

Table 1 — Inhibition zone (mm) of extracts against the sample plant pathogenic bacteria by well and disc method

	Xanthomonas axonopodis pv. *punicae*		*Bacillus pumilus*		*Pseudomonas syringae*	
Mushroom	Well (mm)	Disc (mm)	Well (mm)	Disc (mm)	Well (mm)	Disc (mm)
Podaxis pistillaris	14.97	9.7	40.3	33	29.3	20.8
Phellorina inquinans	22.67	17	31	22	42.7	28
Leucopaxillus giganteus	35	29	45.83	34	44.17	33.67
Antibiotics	52	40	47	36	46	37
SEm	1.32	0.90	0.65	0.99	1.148	0.88
CD (5 %)	4.29	2.95	2.14	3.22	3.74	2.87
CV	8.42	7.48	3.17	6.30	5.66	5.89

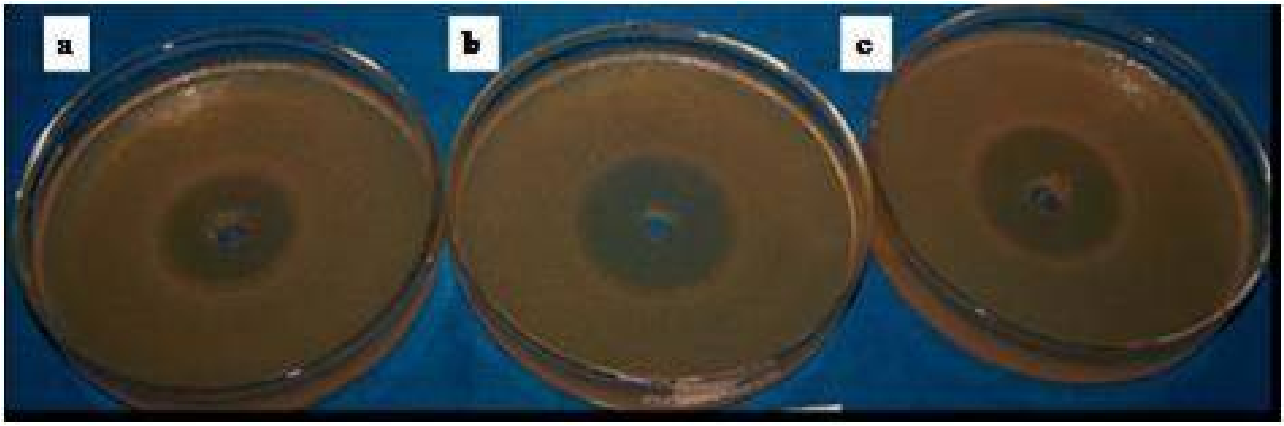

Plate 1 — Zone of inhibition against *Pseudomonas syringae* by; a) *Podaxis pistillaris* b) *Leucopaxillus giganteus*, and c) *Phellorinia inquinans*

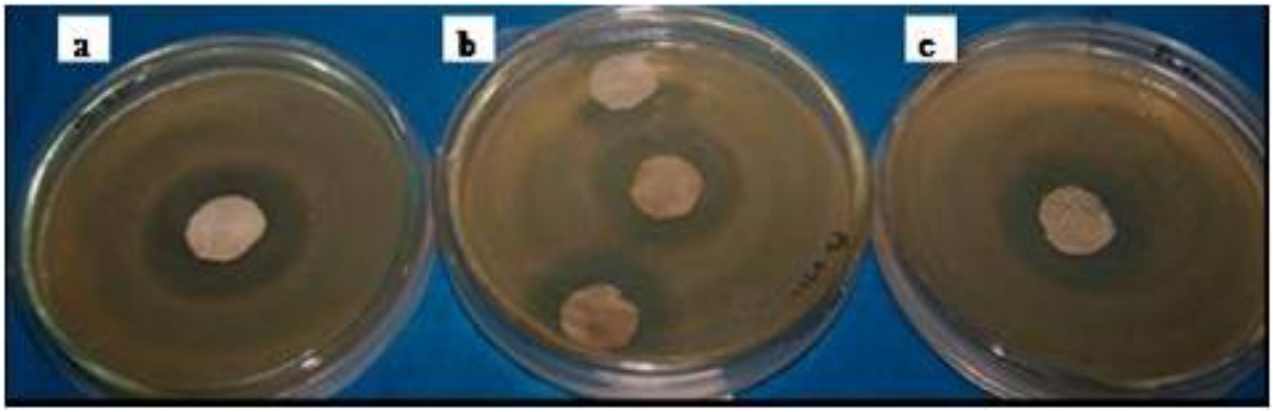

Plate 2 — Zone of inhibition against *Pseudomonas syringae* by; a) *Leucopaxillus giganteus,* b) *Podaxis pistillaris*, and c) *Phellorinia inquinans*

Table 2 — Size of inhibition zone (mm) by ethanol extract of Indian wild mushrooms against the sample plant pathogenic fungi

Plant pathogenic Fungi	Average size of inhibition zone (mm)				SEm	CD (5 %)	CV
	Podaxis pistillaris	*Phellorinia inquinans*	*Leucopaxillus giganteus*	Antibiotics			
Alternaria solani	15	7.5	21	59.83	0.58	1.88	4.47
Aspergillus solani	35	31.33	48	68.4	0.77	2.50	3.36
Aspergillus niger	44.67	39	48	70.53	0.75	2.44	2.97
Colletotrichum graminicola	15.17	17.8	9.6	64.27	0.64	2.09	4.79
Fusarium solani	35	49.87	55	75.57	0.50	1.63	1.86
Fusarium oxysporum	42	56.97	59.47	79.77	1.07	3.48	3.58
Helminthosporiumaydis	36.25	20	55.6	87	0.48	1.58	1.95

inhibition zones by well diffusion and filter paper disc method, respectively, against *P. syringae.*

It is known that in well diffusion method there is a better contact and diffusion of the extracts into the media and organisms while filter paper disc may act as barrier between the extract and the organisms[31]. There may not be proper diffusion and total release of active components adsorbed by the discs into the media. The present results indicate that Gram-positive bacterium (*B. pumilus*,) is more sensitive than Gram-nagative bacteria (*P. syringae* and *X. axonopodis* pv.*punicae*) (Table 1). The sensitivity of Gram-positive bacteria to the antimicrobial agents as compared to Gram-negative bacteria might be explained by the differences in the cell wall structure that can produce differences in antibiotic susceptibility of the cells[32]. It is well known that Gram-negative bacteria possesses an outer membrane and a periplasmic space, both of which are absent in Gram-positive bacteria.

Antifungal activities

The ethanolic extracts of all the three higher fungi showed varying degrees of antifungal activities against the test plant pathogenic fungi (Table 2). *L. giganteus* produced the highest zone of inhibition (59.47 mm) against *F. oxysporum* followed by *P. inquinans* (56.97 mm) while, *P. pistillaris* produced a clear zone of 42.0 mm against *F. oxysporum*. Similarly,*L. giganteus* (55 mm) and *P. inquinans* (49.87 mm) showed higher inhibition zone against *F. solani* while *P. pistillaris* (35 mm) produced lesser inhibition zone against the same fungi (Plate 3). Previous reports revealed the antagonistic effects of *L. tricolor* extracts against *A. fumigatus* and *F. dimerium*[3]. The inhibitory effects of antifungal peptide isolate from *Pleurotus ostreatus* against *Fusarium oxysporum, Mycosphaerella arachidicola* and *Physalospora piricola* were reported in related works[33].

The antifungal activities of *L. giganteus* were generally higher than *P. pistillaris* and *P. inquinans* against *A. niger* and *A. solanium* (Plate 4). For ethanolic extract of *L. giganteus*, inhibition zone of 48 mm was obtained against *A. niger* and *A. solanium.* The inhibition zone decreased significantly to 44.67 and 39 mm for *P. pistillaris* and *P. inquinans,* respectively, against *A. niger* (Table 2). Inhibition zone *of A. solanium* was reducing to 35 and 31.3 mm, when ethanol extract of *P. pistillaris* and *P. inquinans* were applied with the well method, respectively.

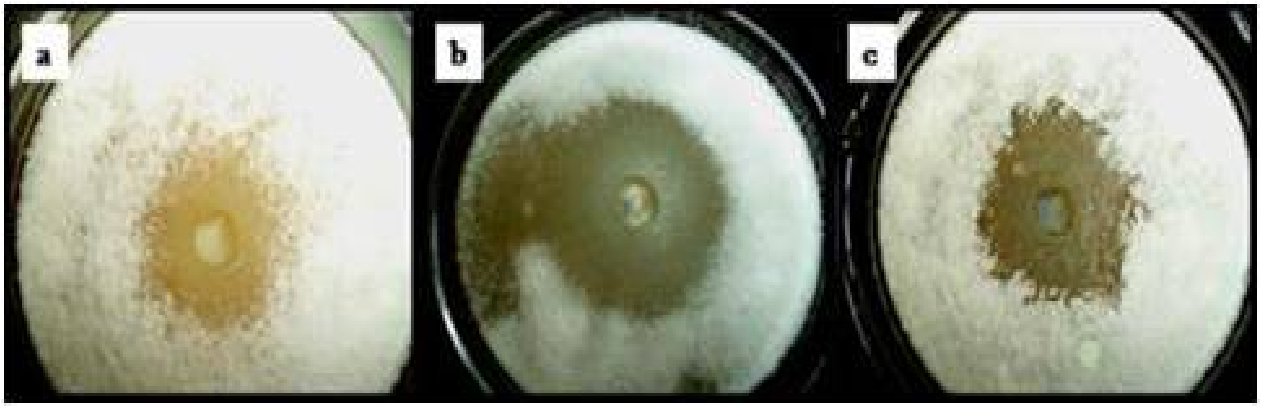

Plate 3 — Zone of inhibition against *Fusarium solani* by; (a) *Podaxis pistillaris*, (b) *Leucopaxillus giganteus*, and (c) *Phellorinia inquinans*

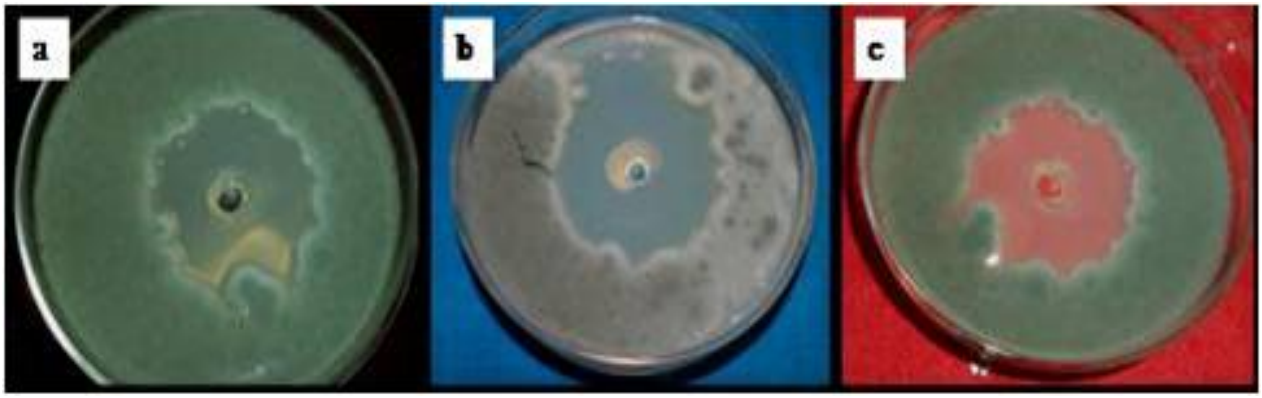

Plate 4 — Zone of inhibition against *Aspergillus solanium* by; a) *Phellorinia inquinans*, b) *Leucopaxillus giganteus*, and c) *Podaxis pistillaris*

The observations were supported by earlier reports that methanolic extract of *Phellinus* showed antifungal activity against five fungal strains; *Penicillium* spp., *A. fumigatous*, *A. niger*, *A. flavus* and *Mucor indicus*[29]. The potent antifungal activity exhibited by *L. giganteus* was in line with the reports in the literature[27]. The growths of all plant pathogenic fungi examined were inhibited by the extracts. The observation suggests that these mushrooms can serve as a potential antifungal agent against plant diseases caused by such organism. The result was supported by the findings in the previous work[1]. The plant pathogenic bacteria showed more sensitivity to the mushroom extracts than fungi. It might be attributed by the differences in the cell wall structure of the microbes that give resistance to the antibiotic[32]. The fungal cell wall structure might be the reason for relatively high resistance towards antimicrobial agents.

Minimum inhibitory concentration (MIC)

It is suggested that at the lowest MIC, the extract will still be effective because of the presence of bio-active compounds[34]. Therefore, higher concentration which consequently poison host cells may not be required. In the present study, the MIC was investigated for 1-20 mg/mL concentrations against three bacteria and seven phytopathogenic fungi separately. The MICs by *L. giganteus* were 1.5, 1.92, and 3.5 mg/mL against *B. pumilus*, *P. Syringae*, and *X. axonopodis* pv. *punicae*, respectively (Table 3). Generally, the least MICs were obtained for *L. giganteus* than *P. Inquinans*, and *P. pistillaris* against the respective plant pathogenic bacteria. In related investigations, the antimicrobial activities of Nigerian higher fungi (*Lycoperdon giganteus*) were studied and the MIC of the ethanolic extracts were 1.25 and 3.25 mg/mL against *E. coli* and *Pseudomonas vulgaris,* respectively[13]. The observed result was comparable with the report of antibacterial and antifungal activities of selected wild mushrooms[24]. In the same instance, the least MICs were observed in case of *L. giganteus* against the respective plant pathogenic fungi than the other mushrooms with the exception against *C. graminicola*

(Table 4). The observed results were in agreement with the reports in the literature[2,13].

The findings revealed that the mushrooms extracts checked in the current work contains potential therapeutic compounds against some of the economically important plant diseases caused by bacteria and fungi, but the MIC against fungi was generally higher than that of bacteria.

Bioactive compounds in the mushroom extracts

Four different assays were carried out to determine the bioactive components of the three Indian wild mushroom extracts. The major bioactive compounds (total phenol, flavonoids, ascorbic acid, β-carotene, and lycopene) concentrations in the mushroom extracts are presented in Table 5. Among the ethanolic extracts of mushrooms, the highest total phenolic compound was found in the fruiting body of *L. giganteus* followed by *P. pistillaris* (Table 5). The lowest total phenolic compound was obtained from the fruiting body of *P. inquinans.* Ascorbic acid and lycopene were also found in small amounts. *L. giganteus* extract contained the highest flavonoid concentration. Ethanol extract of *L. giganteus* was found to have the highest β-carotene and lycopene concentrations as compared to the other two mushrooms extracts. In mushroom extracts, the antioxidant capacity is mainly determined by the amount of phenolic compounds they contain[35]. Higher antioxidant activity of *L. giganteus*, *S. imbricatus*, and *A. arvensis* were observed with total phenol of 6.29, 3.76, and 2.83 mg/g, respectively[36]. This indicates that *L. giganteus* contain high amount of total phenol as compared to other mushrooms, however, this value was still lower when compared to the present findings.

Different wild mushroom species were reported to have antioxidant activity, which was mainly related to their phenolic content[36]. All mushrooms in the current investigation were found to have high concentration of phenol and flavonoid with promising antioxidant properties[37].

Table 3 — Minimum inhibitory concentrations (MIC) of the ethanol extracts of the three wild mushrooms against the test plant pathogenic bacteria

Test organisms	MIC (mg/mL)			SEm	CD (5%)	CV
	Podaxis pistillaris	*Phellorinia inquinans*	*Leucopaxillus giganteus*			
Xanthomonas axonopodis pv. *punicae*	9.5	7.27	3.5	0.18	0.57	5.24
Bacillus pumilus	2.17	3.42	1.5	0.09	0.31	8.15
Pseudomonas syringae	3.67	2.25	1.92	0.10	0.31	7.37

Table 4 — Minimum inhibitory concentrations (MIC) of the ethanolic extracts of three wild mushrooms against the test plant pathogenic fungi

Test organisms	MIC (mg/mL)			SEm	CD (5 %)	CV
	Podaxis pistillaris	*Phellorinia inquinans*	*Leucopaxillus giganteus*			
Alternaria solani	17.25	18.425	14.5	0.16	0.51	1.91
Aspergillus solanium	13.58	15.08	11.75	0.163	0.52	2.42
Aspergillus niger	14.25	16.75	12.42	0.17	0.54	2.35
Colletotrichum graminicola	17.97	14.83	18.17	0.32	1.01	3.79
Fusarium solani	17.93	16.25	13.75	0.36	1.17	4.56
Fusarium oxysporum	15.33	12.17	10.83	0.33	1.04	5.09
Helminthosporium maydis	17.67	16.30	15.5	0.36	1.14	4.34

Results are mean of 4 separate experiments

Table 5 — Major bioactive compounds (mg/g) found in the mushroom extracts

Mushrooms	Total phenols	Flavonoids	Ascorbic acid	*β*-carotene	Lycopene
Podaxis pistillaris	9.58	1.09	0.8	$12.92*10^{-3}$	$15.53*10^{-3}$
Phellorinia inquinans	5.47	1.08	0.83	$9.63*10^{-3}$	$16.61*10^{-3}$
Leucopaxillus giganteus	15.28	2.23	0.97	$14.6*10^{-3}$	$34.92*10^{-3}$
SEm	0.15	0.01	0.03	0.13	0.23
CD (5 %)	0.49	0.02	0.10	0.42	0.72
CV	3.02	1.0	6.89	2.10	2.01

Conclusion

The current study was undertaken to evaluate the antimicrobial activities of ethanolic extracts from fruiting body of three India wild mushrooms (*L. giganteus, P. Inquinans*, and *P. pistillaris*). The results indicated that all the three mushroom extracts exhibited antibacterial and antifungal activity, where plant pathogenic bacteria were more sensitive to the extracts than the fungi. The present investigation revealed that the macrofungi contained a good amount of phenols and flavonoids followed by ascorbic acid, and a very low amount of β-carotene and lycopene. The mushrooms can be used as a potential source of biological control of plant disease that can be integrated in a particular crop protection program. Development of natural antimicrobials from such macrofungi needs further analysis and quantification of individual antimicrobial compounds present in the mushrooms.

Acknowledgment

The authors would like to thank All India Coordinated Mushroom Improvement Project (AICRP) for providing resources and research facilities throughout the investigation.

References

1 Song R Q and Ji H F, Inhibitory effects of eight toxic mushroom strains on growth and germination of *Alternaria alternate*, *J Agric Technol*, 2006, **2**, 39-48.

2 Imtiaj A and Lee T S, Screening an antibacterial and antifungal activities from Korea wild mushrooms, *World J Agric Sci,* 2007, **3**, 316-321.

3 Eren E, Guler P and Kunduz I, Antifungal effects of *Leucopaxillus tricolor* (peck) kühner against to the plant pathogens, *Inter J Bio Pharm Alied Sci,* 2013, **2**, 501-505.

4 Kopczacki P, Gumulka M and Masnyk M, Synthesis and anti-feedant properties of N-benzoylphenylisoserinates of *Lactarius* sesquiterpenoid alcohols, *J Phytochem*, 2001, **58**, 775-787.

5 Quang D N, Hashimoto T and Asakawa Y, Inedible mushrooms: A good source of biologically active substances, *The Chem Record,* 2006, **6**, 79-99.

6 Lindequist U, Niedermeyer T H J and Julich W D, The pharmacological potential of mushrooms, *J Evid Based Complement Altern Med,* 2005, **2**(3), 87-92.

7 Miller H R and Miller O K, A field guide to edible and inedible fungi, *North American Mushrooms* (Falcon Guide) (Guilford, Connecticut), 2006.

8 Liu R H, Potential synergy of phytochemicals in cancer prevention: Mechanism of action, *J Nutr*, 2004, **134**, 3479S-3485S.

9 Munjal R L and Bahl N, A brief description of *Phellorainia inquinans* and also of *Podaxis pistillaris*, *Indian Hortic*, 1973, **17**, 21-22.

10 Sharma Y K and Doshi A, Some studies on an edible wild fungus *Phellorinia inquinans* Ber, Rajasthan, India, *Mushroom Res*, 1996, **5**, 51-53.

11 Zoberi M H, Some Edible mushrooms from Nigeria, *Nigeria Fields,*1978, **38**, 81- 90.

12 Alexopoulous C J, Mims C W and Blackwell M, Introductory Mycology, 4th edn, (John Wiley and Sons, New York Chichester, Brisbane, Toronto and Singapore), 1996.

13 Jonathan S G and Fasidi I O, Antimicrobial activities of two Nigerian edible macro-fungi *Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon giganteum* (Pers.), *African J Biomed Res,* 2003, **6**, 85-90.

14 Stoke J E and Ridgway G L, *Clinical Bacteriology*, 5th edn, (London, 1980), 35-50.

15 Ali-Shtayeh M S, Yaghmour R M, Faidi Y R, Salem K A and Nuri M A, Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area, *J Ethnopharm,* 1998, **60**, 265-271.

16 Tepe B, Daferera D, Sokmen A, Sokmen M and Polissiou M, Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae), *Food Chem,* 2005, **90**, 333-340.

17 Oboh I E, Akerele J O and Obasuyi O, Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta burm.f.* (malvaceae), *Trop J Pharm Res*, 2007, **6**, 809-813.

18 Vamanu E, *In vitro* antimicrobial and antioxidant activities of ethanolic extract of lyophilized mycelium of *Pleurotus ostreatus* PQMZ 91109, *Molecules,* 2012, **17**, 3653-3671.

19 Hirasawa M, Shoujii N, Neta T, Fukushima K and Takada K, Three kinds of antibacterial substances from *Lentinus edobes* (Berk) Sing, (Shitake, an edible mushroom), *Int J Antimicrob Agents*, 1999, **11**, 151-157.

20 Singleton V L and Rossi J A, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am J Enol Vitic,* 1965, **16**, 144-158.

21 Jia Z, Tang M and Wu J, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem,* 1999, **64**, 555-559.

22 Nagata M and Yamashita I, Simple method of simultaneous determination of chlorophyll and carotenoid in tomato fruit, *J Japan Food Sci Technol*, 1992, **39**, 925-928.

23 Klein B P and Perry A K, Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United State, *J Food Sci*, 1982, **47**, 941-945.

24 Nedelkoska D N, Pancevska N A, Amedi H, Veleseka D, Ivanova E, Karadelev M, *et al.*, Screening of antibacterial and antifungal activities of selected Macedonian wild mushrooms, *J Nat Sci*, 2013, **124**, 333-340.

25 Mau J L, Tsai S Y, Tseng Y H and Huang S J, Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill, *LWT-Food Sci Technol*, 2005, **38**, 589-597.

26 Ren G, Zhao Y P, Yang L and Fu C X, Anti-proliferative effect of clitocine from the mushroom *Leucopaxillus giganteus* on human cervical cancer HeLa cells by inducing apoptosis, *Cancer Lett,* 2008, **262**, 190-200.

27 Barros L, Calhelha R C, Vaz J A, Ferreira I C F R, Baptista P and Estevinho L M, Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts, *Eur Food Res Technol,* 2007, **225**(2), 151-156.

28 Sittiwet C and Puangpronpitag D, Anti-*staphylococcus aureus* activity of *Phellinus igniarius* aqueous extract, *Int J Pharm,* 2008, **4**, 503-505.

29 Balakumar R, Sivaprakasam E, Kavitha D, Sridhar, D and Kumar J S, Antibacterial and antifungal activity of fruit bodies of *Phellinus* mushroom extract, *Int J Biosci,* 2011, **1**, 72-77.

30 Al-Fatimi M A A, Jansen W D R and Lindequist U, Bioactive components of the traditionally used mushroom *Podaxis pistillaris, Evid based Complement alternat Med,* 2006, **3**, 87-92.

31 Toda M, Okubu S, Ikigai H, Suzuki T, Suzuki Y and Shimamura T, The Protective activity of tea against infection by *Vibro cholera, J Appl Bacteriol,*199, **70**, 109-112.

32 Walsh F M and Amyes S G B, Microbiology and drug resistance mechanisms of fully resistant pathogens, *Current Trends,* 2004, **7**, 439-444.

33 Chu K T, Xia L and Ng T B, Pleurostrin, an antifungal peptide from the oyster mushroom, *Peptides,* 2005, **26**, 2098-2103.

34 Danielli I, Antimicrobial activities of *Aframomium* species, *J Pharm,* 1957, **16**, 470-472.

35 Jayakumar T, Thomas P A, Sheu J R and Geraldine P, *In-vitro* and *in-vivo* antioxidant effects of the oyster mushroom *Pleurotus ostreatus, Food Res Int,* 2011, **44**, 851-861.

36 Barros L, Ferreira M J, Queiros B, Ferreira C F R and Baptista P, Total phenols, ascorbic acid, beta-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities, *Food Chem,* 2007, **103**, 413-419.

37 Ramesh C and Pattar M G, Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, *India Pharm Res,* 2010, **2**, 107-112.