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Evaluation of phenol content, antibacterial and antioxidant potential of six polypore fungi

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Abstract

This study focuses on antioxidant activities (DPPH free radical scavenging), total phenolic content and antibacterial activities of selected six Polypore fungi belonging to the family Polyporaceae, collected from different regions of forest areas in the Purulia district, West Bengal, India. The phenol content of the samples ranges from 1.8404 ± 0.125747 µg/ml to 11.2608 ± 0.947625 µg/ml. Folin-Ciocalteu reagent is used to measure the phenolic content and its Gallic acid equivalents have been calculated. Higher free radical scavenging activity is observed in lower IC50 (50% inhibition concentration) values. IC50 values range from 49.84 µg/ml to 3993.62 µg/ml. Our results reveal that fungi having higher concentration phenolic content have positive effects against pathogenic bacteria. It shows better antibacterial and antioxidant activities.

Key Words: Antioxidant, Antibacterial, Phenol content, Macrofungi, Polyporaceae

1. Introduction

Macro fungi are essential for antibacterial compounds to survive in their natural environment. Antimicrobial compounds can be extracted from plants [1] and many basidiomycetes species and some of them have potential effects on humans [2]. Many antibiotics that are usually used in the clinical pathway, are extracted from fungi and actinomycetes. During the last few years, several pathogenic microorganisms developed resistance against the available antibiotics. Infections caused by *Candida* sp., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* sp., *Enterococcus* sp. and *Escherichia coli* create multidrug-resistant (MDR). Consequently, we are looking for new antibiotics with novel mechanisms due to emerging drug-resistant pathogenic variants [3-5]. Antimicrobial activities of basidiomycetes fungus from different countries were emphasized. As part of a screening programme designed to assess the antimicrobial activity of basidiomycetes, 317 isolates representing 204 species were collected in Spain and subjected to testing against a variety of human clinical pathogens [6]. In a study, 103 isolates of basidiomycetes fungi belonging to 84 species were found in various locations of Brazil among which 14% of macrofungal extracts were effective against one or more microorganisms [7]. Seventy-five per cent of tested polypore fungi exhibit potent antimicrobial activity, and these could be a valuable resource to produce new antibiotics. Numerous substances derived from these fungi generate antiviral, cytotoxic, and anticancer compounds [8]. The phenolic compounds have antibacterial activity against diverse pathogenic bacteria [9]. Secondary metabolites are beneficial to health and many of them attributes antioxidant, antimicrobial, anticancer, cholesterol-lowering and immunostimulatory properties [10-12]. Phenolics shows strong antioxidant activity [13]. It works as an effective radical scavenger and offers protection from UV radiation or pathogen aggression [14].

In this study, six wild polypore fungi viz *Pycnoporus sanguineus*, *Microporus xanthopus*, *Cubamyces flavidus*, *Lentinus polychrous*, *Cerrena hydnoides* and *Coriolopsis occidentalis*, from Purulia district, have been examined for their phenolic content, antibacterial capabilities, and DPPH activities. We report that relatively higher phenolic contents show better antibacterial activity and some wild polypore fungi involve better free radical compounds.

2. Materials and Methods

Test sample and organism: We have studied the antimicrobial activity, total phenolic content, and DPPH assay from six isolates of basidiomycetes fungus (Plate: 1) belonging to the family Polyporaceae. Antibacterial activity of *Pycnoporus sanguineus* (L.) Murrill, *Microporus xanthopus* (Fr.) Kuntze, *Cubamyces flavidus* (Lév.) Lücking, *Lentinus polychrous* Lév., *Cerrena hydnoides* (Sw.) Zmitr., *Coriolopsis occidentalis* (Klotzsch) Murrill; were tested against one gram-positive pathogenic microorganism *Enterococcus faecalis* (MCC 3037) and seven gram-negative pathogenic bacteria *E. coli* (MCC 3099), *Pseudomonas aeruginosa* (MCC 4242), *Eneterobacter cloacae* (MCC 3111), *Proteus mirabilis* (MCC 3895), *Salmonella enterica* (MCC 4378), *Chromobacterium violaceum* (MCC 2216), and *Escherichia fergusonii* (MCC 4329).

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Extraction procedure for antimicrobial test: Six polypore fungi have been collected from different areas of the forest region in Purulia District, West Bengal. The samples are dried at 35°C in a hot air oven for seven days. Completely dried samples are powdered using a grinder separately. One gram of each powder sample was mixed with 12 ml of each of the solvents-methanol, Dimethyl sulfoxide (DMSO), and cocktail [15-16] (dH2O: ethyl alcohol: methyl alcohol: acetone: chloroform in 1:2.5:2.5:2:2 ratio) in separate airtight conical flasks for 48 hours at 50°C in a rotary incubator shaker for homogenization. Each sample was rubbed separately in a mortar pestle and the solvent extracts were kept separately. Extracts were centrifuged at 8000 rpm for 20 minutes and supernatants were collected separately.

Agar well diffusion method: Antimicrobial activities of test fungi were carried out by well diffusion technique following [17]. Fresh bacterial cultures (100 µl) were pipetted and placed at the centre of sterile petriplates containing media (Luria-Bertani with Kanamycin media was used for *Salmonella enterica* and *Chromobacterium violaceum* and nutrient agar for rest 6 bacterial strains) and spread over petriplates using the spread plate technique. After spreading the fresh bacterial culture, seven wells were prepared (one for control and six for test samples) with the help of a sterilized cork borer. Fungal extracts (250 µl) were added to each well and Petri plates were incubated for 24 hours at 37°C temperature.

Gallic acid standard solution preparation: Standardization of gallic acid was measured according to Hayet *et al.* [18]. Here 0.100 mg of gallic acid dissolves in 10 ml of 80% methanol and then the volume is made up to 100 ml with distilled water in a 100 ml volumetric flask. This gallic acid stock solution was diluted with distilled water to create calibration standard solutions of 20, 40, 60, 80, and 100 µg/ml. One ml of 10% FCR, 10 ml of 7% Na2CO3 (Sodium carbonate), and 4 ml of distilled water were added to each solution. The precursor solutions were allowed to settle for 1 h in dark at room temperature. Finally, the absorbance of these solutions was measured in a UV-vis spectrometer (Shimadzu UV-1800) at 750 nm and the graphical presentation is shown in Fig1.

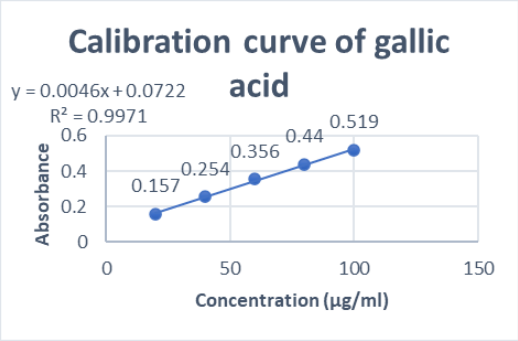

Fig. 1: Linearity curve of gallic acid solution

Estimation of the total phenolic content of the samples: 1 gm of each of the powdered samples of polypore fungi was mixed with 10 ml of methanol in separate airtight conical flasks. Conical flasks were kept in the water bath for two days at 45°C. Then each mixture was ground in a mortar pestle, and centrifuged at 4000 rpm for 20 minutes. The supernatants were collected separately and 100 µl of each of the extract solutions was added with one ml of ten times diluted FCR. After five minutes, 10 ml 7% Na2CO3 was added to each of the test tubes. Each experiment was repeated in triplicate. Finally, the absorbance is measured at 765 nm with the help of Uv-vis spectrophotometer.

Extraction procedure for DPPH assay: 500 mg of each of the powdered samples was mixed with 10 ml of methanol in an airtight container and kept the mixture in a hot air oven at 45°C for 48 hours. The ground samples were centrifuged at 4000 rpm for 20 minutes. 1 ml of each of the supernatants was considered in separate test tubes and diluted up to 10 ml with the same solvent (Methanol).

2,2-Diphenyl-1-picrylhydrazyl assay method: Free radical scavenging activities of methanolic extracts were measured using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay [19]. We have taken various concentrations (50, 100, 150, 200, and 250 µg/ml) of the methanolic extracts of samples. 0.75 µl stock solutions of DPPH (1.3 mg/ml in methanol) were added to each test tube. Test tubes were shaken well and kept in a dark condition for 30 minutes at room temperature. The absorbance was measured at 517 nm using Uv-vis spectrophotometer. 98% methanol was used as blank and the percentage of DPPH free radical scavenging was measured by the following equation [20-22].

% Inhibition = {(Z Control – Z Sample) / Z Control} x 100

Z Control - Absorbance of the control sample, (Methanol + DPPH)

Z Sample - Absorbance of Sample in different concentrations, (Sample + DPPH)

IC50 (50% inhibition concentration) values were calculated from the % inhibition.

3. Results

Antibacterial activity: A total of six polypore fungi have been selected to observe their antimicrobial activity against one gram-positive [*Enterococcus faecalis*, (MCC 3037)] and seven gram-negative bacteria [E. coli, (MCC 3099),

Pseudomonas aeruginosa (MCC 4242), *Eneterobacter cloacae* (MCC 3111), *Proteus mirabilis* (MCC 3895), *Salmonella enterica* (MCC

4378), *Chromobacterium violaceum* (MCC 2216), *Escherichia fergusonii* (MCC 4329)]. We observed that the DMSO extract of fungi *Pycnoporus sanguineus* shows sensitivity against bacterial strain *Enterococcus faecalis* (MCC 3037), and *Escherichia fergusonii* (MCC 4329), Cocktail extraction of fungus *Pycnoporus sanguineus* shows inhibitory effect against bacterial strain *Chromobacterium violaceum* (MCC 2216). DMSO extract of *Microporous xanthopus* shows inhibitory results against bacterial strain *Eneterobacter cloacae* (MCC 3111), and cocktail extract of *Microporous xanthopus* shows sensitive results against bacterial strain *Escherichia fergusonii* (MCC 4329). DMSO extract of, *Cubamyces flavidus* has a potential impact against bacterial strain *Chromobacterium violaceum* (MCC 2216). Cocktail extract of *Lentinus polychrous* shows inhibition against bacterial strain *Enterococcus faecalis* (MCC 3037) (Table 1). DMSO extract of *Pycnosporus senguineus* shows the zone of inhibition (25.644 ± 0.381222) against pathogenic bacteria *Enterococcus faecalis* (MCC 3037) and the zone of inhibition (20.098 ± 0.503954) against *Escherichia fergusonii* (MCC 4329). The cocktail extract of *P. senguineus* was effective (10.636 ± 0.396396) against the bacterial strain *Chromobacterium violaceum* (MCC 2216)

Test fungi	**Solvents**	**Bacterial strains**							
		MCC 4242	MCC 3111	MCC 3037	MCC 3895	MCC 4378	MCC 2216	MCC 4329	MCC 3099
Pycnoporus sanguineus	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	-	+	-	-	-	+	-
	COCKTAIL	-	-	-	-	-	+	-	-
Microporus xanthopus	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	+	-	-	-	-	-	-
	COCKTAIL	-	-	-	-	-	-	+	-
Cubamyces flavidus	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	-	-	-	-	+	-	-
	COCKTAIL	-	-	-	-	-	-	-	-
Lentinus polychrous	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	-	-	-	-	-	-	-
	COCKTAIL	-	-	+	-	-	-	-	-
Cerrena hydnoides	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	-	-	-	-	-	-	-
	COCKTAIL	-	-	-	-	-	-	-	-
Coriolopsis occidentalis	METHANOL	-	-	-	-	-	-	-	-
	DMSO	-	-	-	-	-	-	-	-
	COCKTAIL	-	-	-	-	-	-	-	-

Table 1: The antimicrobial activities of six macrofungal extracts against eight pathogenic bacteria. The '+' sign indicates a positive result (with a zone of inhibition) & '-' sign indicates a negative result (without a zone of inhibition)

DMSO extract of *Microporus xenthopus* is effective (12.38 ± 0.424382) against the bacteria strain *Eneterobacter cloacae* (MCC 3111) and cocktail extract shows effectiveness (19.15 ± 0.221811) against microbial strain *Escherichia fergusonii* (MCC 4329). DMSO extract of, *Cubamyces flavidus* inhibits the growth (13.458 ± 1.021112) of bacterial strain *Chromobacterium violaceum* (MCC 2216) and cocktail extract of *Lentinus polychrous* also inhibits the growth (12.658 ± 0.261859) of bacterial strain *Enterococcus faecalis* (MCC 3037) (Table 2).

Name of fungi	**Concentration of extraction (1g/12ml)**	**Bacterial strain**	**Zone of inhibition (mm)**
Pycnoporus sanguineus	DMSO	MCC 3037	25.644 ± 0.381222
		MCC 4329	20.098 ± 0.503954
	COCKTAIL	MCC 2216	10.636 ± 0.396396
Microporus xanthopus	DMSO	MCC 3111	12.38 ± 0.424382
	COCKTAIL	MCC 4329	19.15 ± 0.221811
Cubamyces flavidus	DMSO	MCC 2216	13.458 ± 1.021112
Lentinus polychrous	COCKTAIL	MCC 3037	12.658 ± 0.261859

Table 2: The zone of inhibition with standard deviation value of Pathogenic bacteria against 7 positive test samples

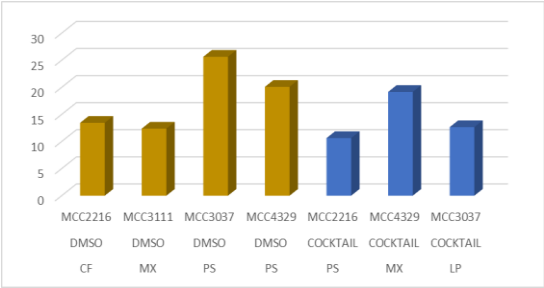

Fig 2: Graphical representation of bacterial strain with their zone of inhibition (in mm) in different solvent (PS: *Pycnoporus sanguineus*, MX: *Microporus xanthopus*, CF: *Cubamyces flavidus*, LP: *Lentinus polychrous*)

Total phenolic content: The phenolic content (Table 3) of the test sample was as follows: *Lentinus polychrous* (11.2608 ± 0.947625)> *Pycnoporus sanguineus* (10.75357 ± 0.502006)> *Cubamyces flavidus* (7.927467 ± 1.972585)> *Microporus xanthopus* (6.5506 ± 0.763484)> *Coriolopsis occidentalis* (3.362233 ± 0.204967)> *Cerrena hydnoides* (1.8404 ± 0.125747). *Lentinus polychrous* and *Pycnoporus sanguineus* had shown the highest phenolic content while lowest phenolic content was observed in *Cerrena hydnoides*. It was observed that the higher percentage of phenol content of the samples is more effective against bacterial strain than the lower percentage of phenolic.

Name of sample	**Sample absorbance**	**Phenol concentration (µg/ml)**
Pycnoporus sanguineus	0.123	10.75357 ± 0.502006
	0.123	

	0.119	
Microporous xanthopus	0.099	6.5506 ± 0.763484
	0.106	
	0.102	
Cubamyces flavidus	0.110	7.927467 ± 1.972585
	0.117	
	0.099	
Lentinus polychrous	0.122	11.2608 ± 0.947625
	0.129	
	0.121	
Cerrena hydnoides	0.081	1.8404 ± 0.125747
	0.081	
	0.080	
Coriolopsis occidentalis	0.089	3.362233 ± 0.204967
	0.087	
	0.087	

Table 3: Quantitative analysis of the total phenolic content of six macrofungal samples

DPPH free radical scavenging activity: DPPH (2,2- diphenyl-1-picrylhydrazyl) method was used in this study to assess the antioxidant activity of methanolic extract of six different polypore fungi. The result demonstrated that we have considered different concentrations (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml) of methanolic extract from the samples. In most cases, the IC50 value (50% inhibition concentration) is used to demonstrate DPPH scavenging activity. The IC50 value of the test sample was as follows *Cubamyces flavidus* (49.84 µg/ml) > *Coriolopsis occidentalis* (451.38 µg/ml) > *Lentinus polychrous* (697.34 µg/ml) > *Microporus xanthopus* (2220.22 µg/ml) > *Pycnoporus sanguineus* (3483.84 µg/ml) > *Cerrena hydnoides* (3993.62 µg/ml). In this study, *Cubamyces flavidus* performed the highest antioxidant property. So, 49.84 µg/ml *Cubamyces flavidus* show 50% free radical scavenging activity and *Cerrena hydnoides* show the lowest free radical scavenging activity (Table 4)

Concentration (µg/ml) of fungal extracts	**Inhibition%**	**DPPH IC50, (µg/ml)**
Pycnoporus sanguineus		
50	0.88±0.26	3483.84
100	1.65±0.31	
150	2.30±0.21	
200	3.01±0.10	
250	3.77±0.09	
Microporus xanthopus		
50	1.24±0.13	
100	1.48±0.23	

150	1.63±0.05	2220.22
200	4.80±0.12	
250	5.07±0.03	
Cubamyces flavidus		
50	4.5±0.50	49.84
100	9.3±0.30	
150	13.56±0.04	
200	17.9±0.35	
250	23.8±0.12	
Lentinus polychrous		
50	3.28±0.40	697.34
100	7.69±0.35	
150	12.4±0.34	
200	14.5±0.5	
250	17.06±0.21	
Cerrena hydnoides		
50	1.03±0.20	3993.62
100	1.74±0.03	
150	2.45±0.06	
200	2.97±0.02	
250	3.52±0.06	
Coriolopsis occidentalis		
50	6.30±0.51	451.38
100	10.76±0.25	
150	18.03±.78	
200	22.04±0.42	
250	28.01±.78	

Table 4: DPPH radical scavenging of the extract from fungi at the different concentration level

Plate 1: Photographs of Polypore Macrofungi: a. *Pycnoporus sanguineus;* b. *Microporus xanthopus;* c. *Lentinus polychrous;* d. *Cerrena hydnoides;* e. *Coriolopsis occidentalis;* f. *Cubamyces flavidus*

4. Discussion

The result of present study shows that Methanolic extraction of this test fungi showed no significant antibacterial activities against pathogenic bacteria. DMSO and cocktail were found to be the best solvents for the solubilization of bioactive components for polypore fungi. The fungi with Phenol content ranges from 6.5506 µg/ml to 11.2608 µg/ml had shown better antibacterial activities. Relatively higher phenolic content of *Pycnoporus sanguineus* and *Lentinus polychrous* may be responsible for antibacterial activity against the gram-positive bacterial strain *Enterococcus faecalis* (MCC 3037). Similarly high phenol content of *Cubamyces flavidus* and *Microporous xanthopus* may be corelated with their antibacterial properties against three gram-negative bacterial strains (Table 2 and Table 3). *Cubamyces flavidus* has shown (49.84µg/ml) the best antioxidant activities, whereas, *Cerrena hydnoides* has shown the least (3993.62 µg/ml) activity. Further details metabolite profiling of the test fungi may open a new horizon for discovering drugs against pathogenic bacteria.

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