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Antimicrobial Activities Of Basidiocarps Of Wild Edible Mushrooms Of West Bengal, India

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Abstract: In this study, antimicrobial activity of the methanolic extract of edible mushrooms of West Bengal, India, such as *Agaricus campestris* L., *Amanita vaginata* (Bull.) Lam., *Armillaria mellea* (Vahl) P. Kumm., *Astraeus hygrometricus* (Pers.) Morgan, *Auricularia auricula* (L.) Undrew., *Auricularia* sp., *Calocybe indica* Purkayastha and Chandra, *Fistulina hepatica* (Schaeff.) With., *Hygrophorus miniatus* (Fr.) Fr., *Lepiota procera* (Scop.) Gray, *Lepiota* sp., *Pleurotus djamor* var. *djamor* (Rumph. ex Fr.) Boedijn, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Pleurotus sajor-caju* (Fr.) Singer, *Lentinus squarrosulus* (Mont.) Singer ex Pegler, *Polyporus grammocephalus* Berk., *Ramaria botrytis* (Pers.) Ricken, *Russula albonigra* (Krombh.) Fr., *Russula delica* Fr., *Russula laurocerasi* Melzer, *Russula lepida* Fr., *Schizophyllum commune* Fr., *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R. Heim, *Termitomyces microcarpus* (Berk. and Broome) R. Heim, *Tricholoma giganteum* Massee, *Tricholoma lobayense* R. Heim, *Tricholoma* sp., *Tricholoma crassum* Sacc. and *Volvariella volvacea* (Bull.) Singer, were investigated.
Key words: Mushroom, Antimicrobial, *Lentinus squarrosulus, Russula albonigra, Tricholoma giganteum.*

INTRODUCTION

Mushrooms have been used as a part of regular diet for their nutritional and medicinal values mostly by the ethnic group of Asian people from time immemorial. They contain minerals, vitamins and nutritive compounds, proteins, polysaccharide and have a low fat content [1]. Their therapeutic interest in promoting human health is known for thousands of years. Modern scientific investigations showed that mushrooms have immense potentiality against a wide range of human ailments such as cardioprotective [2], hepatoprotective [3, 4], chemopreventive [5, 6], immunomodulatory [7], and also strong free radical scavenging activity [8, 9, 10, 11, 12]. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents [13]. Because of these burning problems, the scientific community, in search of new therapeutic alternatives, has studied many kinds of mushrooms, which are a nutritionally functional food, and a source of physiologically beneficial and nontoxic medicines [14]. Several compounds

extracted from mushrooms revealed antifungal and anti bacterial activity [7, 15].

Anti microbial drugs have long been used for prophylactic and therapeutic purposes. Resistance of microorganisms to antibiotics has created an immense clinical problem in the treatment of infectious diseases [16]. Hence, the current investigation was undertaken to screen some common wild edible mushrooms of West Bengal for their antimicrobial activities.

MATERIALS AND METHODS

Fungal material

Thirty kinds of mushroom basidiocarps were collected from different corners of West Bengal and used for screening of anti microbial activity. The basidiocarps of *Hygrophorus miniatus* (Fr.) Fr., *Ramaria botrytis* (Pers.) Ricken, *Fistulina hepatica* (Schaeff.) With., *Armillaria mellea* (Vahl) P. Kumm., were collected from hilly regions of West Bengal, *Calocybe indica* Purkayastha and Chandra, *Tricholoma crassum* Sacc., *Schizophyllum commune* Fr., *Auricularia auricula* (L.) Undrew., *Auricularia* sp., *Termitomyces eurhizus* (Berk.) R. Heim, *Polyporus grammocephalus* Berk., *Tricholoma giganteum* Massee, *Lentinus squarrosulus* (Mont.) Singer ex Pegler, *Tricholoma* sp., *Termitomyces microcarpus* (Berk. and Broome) R. Heim, *Volvariella volvacea* (Bull.) Singer, *Lepiota procera* (Scop.) Gray, *Pleurotus djamor* var. *djamor* (Rumph. ex Fr.) Boedijn, *Pleurotus sajor-caju* (Fr.) Singer, *Agaricus campestris* L., *Pleurotus ostreatus* (Jacq.) P. Kumm., *Tricholoma lobayense* R. Heim, *Lepiota* sp., *Termitomyces clypeatus* R. Heim, from Gangetic plains of West Bengal, and *Astraeus hygrometricus* (Pers.) Morgan, *Russula laurocerasi* Melzer, *Russula albonigra* (Krombh.) Fr., *Russula delica* Fr., *Russula lepida* Fr., *Amanita vaginata* (Bull.) Lam., from lateritic region of West Bengal.

The specimens were identified with the help of standard literatures like Purkayastha and Chandra (1985), Singer (1986), Roy and De (1996), and Das and Sharma (2005) [1, 17, 18, 19].

The voucher specimens have been deposited in the laboratory of Molecular and Applied Mycology and Plant Pathology, Department of Botany, University of Calcutta.

Extraction

Fresh mushrooms were randomly selected into three samples of 150 g each and air-dried in an oven at 40°C for 48 h. Dried powdered mushroom sample was extracted by stirring with 200 ml of methanol at 30°C for 24 h at 150 rpm and filtering through Whatman No. 4 filter paper. The residue was then extracted twice with another 200 ml of methanol as described above. The total extract was then rotary evaporated to dryness at 40°C and redissolved in methanol to a concentration of 10 mg/ml and stored at -20°C for further use [8].

Microorganisms

All the test microorganisms *Staphylo coccus aureus* MTCC CODE 96, *Proteus vulgaris* MTCC CODE 426, *Candida albicans* MTCC CODE 183, *Bacillus cereus* MTCC CODE 1306, *Escherichia coli* MTCC CODE 68, *Pseudomonas aeruginosa* MTCC CODE 8158, *Bacillus subtilis* MTCC CODE 736, were obtained from the culture collection of the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

Screening of antimicrobial activity of the methanolic extracts

Antimicrobial activities of the methanolic extracts of different mushrooms were determined by the disc diffusion method. *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were incubated at 37 ± 0.1 °C for 24 hours by inoculation in nutrient broth. *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus cereus* were incubated at 37 ± 0.1 °C for 24 hours by inoculation in Growth media 3 (GM 3) broth (Peptone 0.5 %, Sodium chloride 0.5 %, Beef extract 0.1 %, Yeast extract 0.2 %). *Candida albicans* was incubated at 28 ± 0.1 °C for 48 hours by inoculation in YEPD broth. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 Mc Farland turbidity standard tubes. Nutrient agar, GM 3 agar and YEPD agar (20 ml) were poured into each sterilised Petri dish (10 × 90 mm diameter) after injecting cultures (100 µl) of bacteria and yeast. The media were distributed in Petri dishes homogenously. For the investigation of antimicrobial activity, the methanolic extracts obtained from dried edible mushrooms were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10% and sterilized by 0.22 micron membrane filter [20]. 10 µl aliquots of each extract was applied on sterile paper discs of 5 mm diameter (1 mg /disc), placed over the surface of the medium, and incubated at 37 °C for 24 hours. At the end of the incubation period, inhibition zones formed on the medium were evaluated by measuring the diameters of the zones of inhibition in millimeter. Inhibitory activity of the DMSO was also tested as negative control. Studies were performed in triplicate.

Standard antibiotic discs Tetracycline (30 µg), Amphotericin (20 µg), and Ceftriazone (30 µg) were used as positive control.

Determination of Minimal inhibitory concentration (MIC)

The MIC study was aimed to find out the lowest concentration of the sample that inhibits the growth of the test organisms. The test was carried out using filter paper disc method with varying concentrations of the extracts placed on bacteria and yeast seeded plates. Plates were incubated at 37 °C for 24 h and the MIC recorded as the lowest concentration at which no growth was observed.

RESULTS AND DISCUSSION

The methanolic extracts of the basidiocarps of thirty different wild edible mushrooms were tested against three Gram-positive (*B. cereus*, *B. subtilis*, *S. aureus*), three Gram-negative (*P. vulgaris*, *P. aeruginosa*, *E. coli*) bacteria and a Yeast (*C. albicans*) by the disc diffusion method. All the extracts showed different degree of antimicrobial activity at a concentration of 500 µg/disc against the test organisms [Table 1]. Among all the microbes, *P. vulgaris* showed highest sensitivity towards mushrooms extracts (21 different mushroom extracts) followed by *P. aeruginosa* (19 different mushroom extracts). The degrees of susceptibility of the microbes were in the order: *P. vulgaris* > *P. aeruginosa* > *E. coli* > *B. cereus* > *C. albicans* > *B. subtilis* = *S. aureus* [Figure 1].

Furthermore, when we compared the efficacy of the extracts having antimicrobial activity, it was noticed that four mushrooms namely *C. indica*, *P. djamor* var. *djamor*, *R. botrytis* and *T. clypeatus* showed activity against only *P. aeruginosa*, *C. albicans*, *P. vulgaris* and *P. aeruginosa*, respectively. Similarly nine mushroom extracts showed activity against two microbes, seven extracts against three microbes, five extracts against four microbes, two extracts against five microbes and only three mushroom extracts namely *L. squarrosulus*, *R. albonigra* and *T. giganteum* showed antimicrobial activity against all the test microbes [Figure 2, Table 1].

The MIC is the lowest concentration of a substance at which it plays to inhibit the growth of the target microorganisms. We have studied 50 – 200 µg/disc of the methanolic extracts of *L. squarrosulus*, *R. albonigra* and *T. giganteum* against all the test organisms. Low minimum inhibitory concentration was observed in case of *L. squarrosulus* methanolic extract [Table 2], highlighting the potential of this mushroom as an excellent source of antimicrobial agent.

We have already reported potent anticancer, hepatoprotective activities of other edible mushrooms [21, 22]. The results of the present study strengthened the outcomes of earlier works done by others that showed mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. [23, 24]; *Fomitopsis* sp. [25]; *Boletus* sp. [26]; *Cortinarius* sp. [27]; *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* [28]; *Pleurotus tuber-regium* [29]; *Amanita caesarae*, *Armillaria mellea*, *Chroogomphus rutilus*, *Clavariadelphus truncates*, *Clitocybe geotropa*, *Ganoderma* sp., *Ganoderma carnosum*, *Hydnum repandum*, *Hygrophorus agathosmus*, *Lenzites betulina*, *Leucoagaricus pudicus*, *Paxillus involutus*, *Polyporus arcularius*, *Rhizopogon roseo*, *Sarcodon imbricatus*, *Suillus collitinus*, *Trametes versicolor*, *Tricholoma auratum*, *Tricholoma fracticum* [30]; *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* [31]; *Russula delica* [32]; *Pleurotus eryngii* var. *ferulae* [33]; *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius delicious* and *Phellinus hartigii* [34]; *Lactarius indigo* [35] and *Stereum ostrea* [36] contain a wide range of antimicrobial activity. In conclusion, most of the basidiomycetes species studied had a diverse range of antimicrobial activity while only *L. squarrosulus*, *R. albonigra* and *T. giganteum* methanolic extract showed activity against all the test microorganisms. MIC of methanolic extract of *L. squarrosulus* was lower than that of *R. albonigra* and *T. giganteum* against all the test organisms. We have also reported earlier that *L. squarrosulus* has a potential free radical scavenging activity [37]. The result of the former and current study may suggest that the basidiocarp of *L. squarrosulus* is a source of pharmacologically active substances having diverse therapeutic applications. Bio-assay guided isolation of active principle is currently underway to characterize the antimicrobial compound of this interesting taxon.

Table 1: Antimicrobial activity of methanolic extract of different specimens.

Pathogenic strains / **Samples**	***Staphylococcus aureus***	***Bacillus cereus***	***Bacillus subtilis***	***Proteus vulgaris***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
Agaricus campestris	-	-	+	+	-	+	+
Amanita vaginata	+	-	-	+	+	-	+
Armillaria mellea	+	-	-	+	+	-	-
Astraeus hygrometricus	-	-	-	+	+	+	+
Auricularia auricula	-	+	-	+	-	+	-
Auricularia sp.	-	+	-	-	-	+	-
Calocybe indica	-	-	-	-	-	+	-
Fistulina hepatica	-	-	-	+	+	-	-
Hygrophorus miniatus	-	+	-	+	-	+	-
Lepiota procera	-	-	+	+	+	+	+
Lepiota sp.	-	-	-	+	-	+	-
Pleurotus djamor var. *djamor*	-	-	-	-	-	-	+
P. ostreatus	+	-	+	+	+	+	-
P. sajor-caju	-	+	-	-	+	-	-
Lentinus squarrosulus	+	+	+	+	+	+	+
Polyporus grammocephalus	-	-	-	+	+	+	-
Ramaria botrytis	-	-	-	+	-	-	-
Russula albonigra	+	+	+	+	+	+	+
R. delica	-	-	+	-	+	+	-
R. laurocerasi	-	-	-	+	-	+	+
R. lepida	+	-	-	-	+	+	+
Schizophyllum commune	-	+	-	-	+	+	+
Termitomyces clypeatus	-	-	-	-	-	+	-
T. eurhizus	-	-	-	+	+	-	-
T. microcarpus	-	+	-	+	-	-	-
Tricholoma crassum	-	+	-	+	-	-	-
T. giganteum	+	+	+	+	+	+	-
T. lobayense	-	-	-	-	-	+	+
Tricholoma sp.	-	+	-	+	-	-	+
Volvariella volvacea	-	+	-	+	+	-	+
Tetracycline	+	+	+	+	+	-	NT
Amphotericin	NT	NT	NT	NT	NT	NT	-
Ceftriazone	+	+	+	+	+	+	NT

Symbols: [+] = Positive zone of inhibition. [-] = No inhibition. [NT] = Not tested.

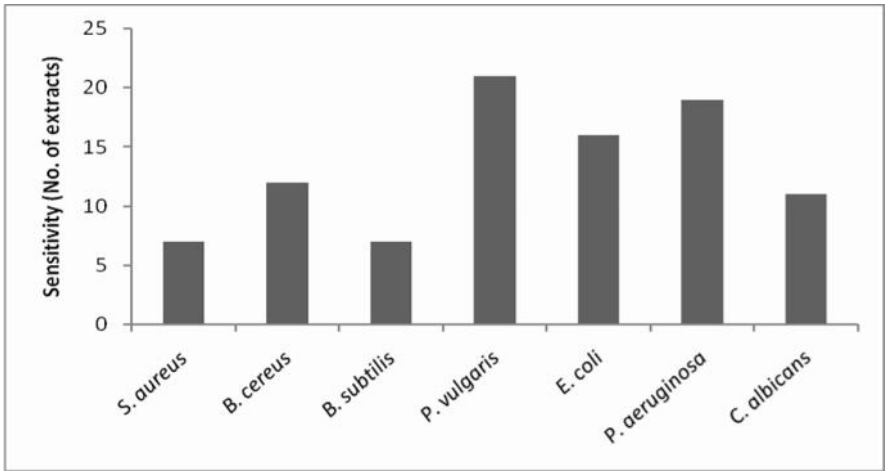

Figure 1: Sensitivity of the test organisms towards thirty different mushroom extracts.

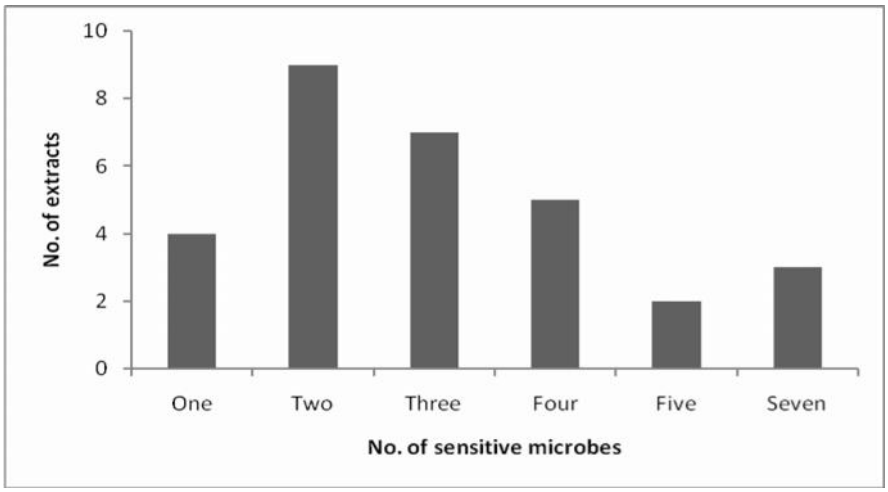

Figure 2: Sensitivity range of different mushroom extracts towards the test pathogenic microorganisms.

Table 2: Minimum inhibitory concentrations (MIC) of the methanolic extract of three potential mushrooms against all test microbes. Results are mean of three separate experiments, each in triplicate.

Test organisms	MIC (µg/disc)		
	Lentinus squarrosulus	***Russula albonigra***	***Tricholoma giganteum***
Staphylococcus aureus	50	75	75
Bacillus cereus	50	75	75
Bacillus subtilis	75	75	75
Proteus vulgaris	75	75	75
Escherichia coli	50	75	75
Pseudomonas aeruginosa	50	75	75
Candida albicans	50	100	75

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