

Wild edible macrofungi consumed by ethnic tribes of Tripura in Northeast India with special reference to antibacterial activity of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn

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Abstract

A survey was conducted to assess the ethnomycological knowledge among the ethnic tribes of Tripura, Northeast India and to assess the antibacterial activity of extracellular mycelial extract of *Pleurotus djamor*. Thirteen wild edible macrofungi were documented from the local markets and forests belonging to eight families under eight genera. *Lentinus* spp., *Pleurotus* spp. and *Termitomyces* spp. were common in the markets. *Termitomyces* spp. and *Tricholoma* spp. were favoured over others as dietary items but were seasonal on their availability. The best antimicrobial activity of extracellular mycelial extract of *P. djamor* was shown against *Ralstonia pickettii* followed by *Bacillus subtilis*. The local demand indicates that commercialization with cultivation of *Lentinus* spp., *Pleurotus* spp. and *Termitomyces* spp. may increase their availability during off-season which will also assist in economic benefits to the local people. The efficacy of extracellular mycelial extract of *Pleurotus djamor* against bacteria was tested being the most commonly available and consumed mushroom from amongst the studied samples. The further study should be directed towards the isolation of bioactive compounds from the extracellular mycelium extracts of *P. djamor*.

Keywords

Wild edible macrofungi
Ethnic tribes, Tripura
Northeast India
Antibacterial
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Introduction

Northeast India is inhabited by several ethnic tribal communities having sound mycological knowledge and a large number of edible mushrooms are used for consumption by these tribal communities. Consumption of such wild edible mushrooms was reported from Assam, Meghalaya, Nagaland and Manipur (Sing and Sing, 1993; Sarma *et al.*, 2010; Tanti *et al.*, 2011; Khaund and Joshi, 2013). Edible mushrooms are a heterogeneous group which includes both Ascomycetes and Basidiomycetes (Purkayastha and Chandra, 1985). Wild mushrooms are an important non-timber forest resource used by mycophilic societies and their mode of utilization has been documented in many countries around the world (Thatoi and Singdevsachan, 2014). Traditional mycological knowledge of most Indian ethnic groups has proven to be widespread and intense, consuming nearly 283 species of wild mushrooms out of 2000 species recorded world over (Purkayastha and Chandra, 1985).

Tripura is one of the states in northeast India inhabited by 19 major tribes. The origin and distribution of ethnic tribes are found to localize in particular area. These ethnic groups are engaged in collection and consumption of mushrooms based on their traditional knowledge. In the present investigation, survey was conducted to collect, document and identify some indigenous mushrooms using morphological and microscopic parameters. Further, the information on indigenous and traditional knowledge regarding use of wild edible mushrooms by the ethnic tribes was collected comprehensively from the mushroom sellers.

The genus *Pleurotus* comprises various edible mushroom species and has important medical and biotechnological properties and environmental applications (Cohen *et al.*, 2002). Of the *Pleurotus* species, *Pleurotus djamor* (Fr.) Boedijn. was reported from India (Srivastava, 2001; Saha *et al.*, 2012). The cultivation of this species was studied (Junior *et al.*, 2010). Antioxidant properties were reported earlier (Saha *et al.*, 2012). The present study is first of its

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Table 1. Wild edible mushrooms collected from various markets and natural habitats in Tripura

Market/Natural Habitat	N Latitude	E Longitude	Altitude (masl)
Lake Chowmuhari Bazar	23°50'31.52"	91°16'55.46"	17
Sepahijala	23°39'49.35"	91°18'20.26"	41
A.D. Nagar	23°47'48.51"	91°16'20.40"	24
Kanchanpur Bazar	24°02'15.18"	91°12'03.96"	64
M.B. Tila Bazar	23°48'39.17"	91°15'57.18"	28
Chowmuhari Bazar	23°44'44.71"	91°16'14.22"	27
Taidu Bazar	23°44'02.29"	91°39'14.43"	66
Mandwi Bazar	23°51'36.87"	91°28'46.61"	78
Udaipur Bazar	23°32'03.40"	91°28'44.22"	28
Vanghmun (Jampui Hill)	23°59'21.41"	91°16'37.04"	638
Jirania Bazar	23°49'06.60"	91°26'51.24"	42
Badharghat	23°48'08.98"	91°16'20.02"	19

kind as far as the mycological knowledge, collection and documentation of wild edible mushrooms from Tripura state is concerned in India. Moreover, the extracellular mycelial extract has not been tested for its activity against bacteria. Thus, we assessed the antibacterial activity of mycelium extract of *Pleurotus djamor*.

Materials and Methods

Sampling of edible mushroom

The population of Tripura is 3.671 million (Census of India, 2011) with the geographical location extending from 22° 56' N 91° 09' E to 24° 32' N 92° 20' E comprising eight districts i.e., West Tripura, Sepahijala, Gomati, South Tripura, Khowai, Dhalai, Unokoti and North Tripura. It covers a total area of 10,491.69 Km². The state has the international border on the northern, southern and western side with the Bangladesh. The average annual rainfall ranges from 1979.6 to 2745.9 mm and the summer temperature falls between 24°C and 36°C. The forest area covers 6292.681 km² mainly consisting of tropical evergreen, semi evergreen and moist deciduous.

The survey was conducted in 12 locations of Tripura (Table 1). The survey for collection and documentation of wild edible mushrooms was conducted in few markets and natural habitats during April-October, 2012 (Figure 1). Collected mushroom samples were packed in sterilized polythene zipped bag and brought to the laboratory for their identification. Dried specimens were also preserved as herbarium material in the Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University. The habitat, colour, shape and size, odour and adaptation to the environment were recorded prior to the preservation of the collected macro fungi. Identification of the specimens was carried out by standard microscopic methods considering various morphological characteristics (Pegler 1977, Purkayastha and Chandra, 1985, Junior *et al.*, 2010).

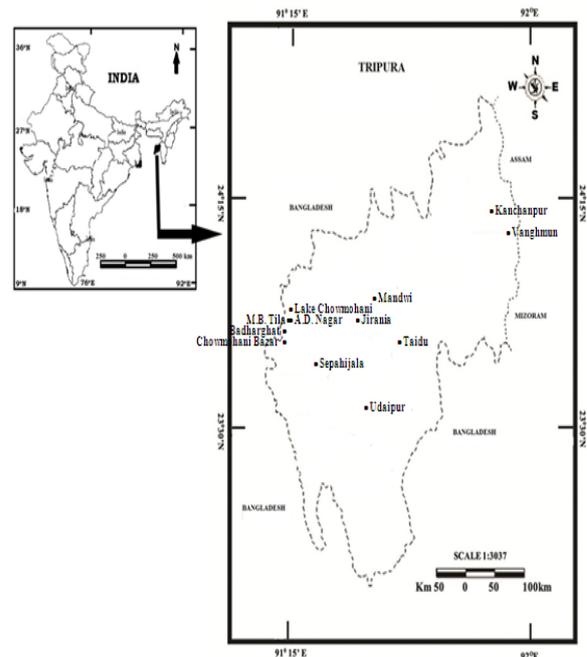


Figure 1. Map of Tripura showing various markets and natural habitats selected for the collection of wild edible mushrooms

Determination of antibacterial activity

Inoculation of the mushroom mycelium of seven days old cultures was done in 100 ml basal synthetic medium broth (BSM) in 250 ml Erlenmeyer flasks and was incubated for 21 days at 25°C in stationary condition. The broth of each submerged mycelium was filtered and the filtrate was used for the antimicrobial activity. Antimicrobial properties were determined by Filter paper disc diffusion method (Bauer *et al.*, 1966). Twenty five microlitre of mushroom mycelium extract was loaded on sterile filter paper discs (5 mm in diameter) and air dried. The 0.1 ml of each bacterial broth was transferred to nutrient agar plates and the discs were placed on plates. Diameters of the zones of inhibition were measured in millimeters. Discs loaded with each extract from the broth BSM medium were considered as control. The mushroom extract was tested against the bacteria procured from Institute of Microbial Technology (IMTECH) Chandigarh. The bacterial species used for investigations were *Bacillus subtilis* (MTCC-619), *Erwinia* sp. (MTCC-2760), *Xanthomonas campestris* (MTCC-2286), *Pseudomonas aeruginosa* (MTCC-424) and *Ralstonia picketti* (MTCC-6280). The results obtained were compared with the standard antimicrobial agents, ketoconazole (100 µg/ml), clotrimazole (25 µg/ml) and streptomycin (100 µg/ml).

Data analysis

The antimicrobial property was assessed after

Table 2. Edible mushrooms with their vernacular names collected from the study sites and their market price

Wild edible mushrooms	Family	Accession Number	Markets	Natural habitat	Price/Kg (INR)	Vernacular name
<i>Craterellus cornucopioides</i>	Cantharellaceae	MCCT 07	Lake Chowmuhani	-	500	-
<i>Lentinus sajor-caju</i>	Polyporaceae	MCCT 04	Lake Chowmuhani and Jirania	Sepahijala	350	<i>Buphangni mikhumu</i>
<i>L. squarrosulus</i>	Polyporaceae	MCCT 03	Lake Chowmuhani and Jirania	AD. Nagar	300	<i>Buphangni mikhumu</i>
<i>Macrolepiota procera</i>	Agaricaceae	MCCT 02	-	AD. Nagar	-	-
<i>P. cornucopiae</i>	Pleurotaceae	MCCT 09	Lake Chowmuhani and Lake Chowmuhani and	-	300	<i>Buphangni mikhumu</i>
<i>P. djamor</i>	Pleurotaceae	MCCT 01	Jirania	-	300	<i>Buphangni mikhumu</i>
<i>P. ostreatus</i>	Pleurotaceae	MCCT 10	Kanchanpur and Jirania	-	400-500	<i>Buphangni mikhumu</i>
<i>P. petalooides</i>	Pleurotaceae	MCCT 08	MB. Tilla	-	450	<i>Buphangni mikhumu</i>
<i>Schizophyllum commune</i>	Schizophyllaceae	MCCT 12	-	Vangmun (Jampui Hill) and Sepahijala	-	-
<i>Termitomyces heimii</i>	Lyophyllaceae	MCCT 13	Chowmuhani Bazar, Mandwi and Kanchanpur	-	750-900	<i>Mikhumu khapolok</i>
<i>T. eurhiza</i>	Lyophyllaceae	MCCT 06	Lake Chowmuhani	-	800-1000	<i>Mikhumu khapolok</i>
<i>Tricholoma giganteum</i>	Tricholomataceae	MCCT 05	Taidu and Udaipur Bazar	-	1000-1200	<i>Khasong</i>
<i>Volvariella media</i>	Pluteaceae	MCCT 14	-	Badharghat	-	-

inoculation with bacteria and zone of inhibition (mm) was noted down after 48 h of incubation. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Diameter of inhibition zone (mm)}}{\text{Total diameter of Petri dish (mm)}} \times 100$$

Results

Edible mushroom of Tripura

Among 12 locations used for collection of the mushrooms, 8 places were markets and 4 were forests. Thirteen wild edible macrofungi were collected from markets and natural habitats which were assigned to eight families under eight genera (Table 2). Of these thirteen mushrooms, eleven were sold in the markets (Figure 2) and five were also collected from their natural habitats. The information collected from the ethnic people regarding mushrooms from the natural sites viz., *Lentinus sajor-caju*, *Volvariella media*, *Lentinus squarrosulus*, *Macrolepiota procera* and *Schizophyllum commune* indicated them to be edible. The market price of these mushrooms varies from market to market (Table 2).

Antibacterial activity

The results showed that the percentage inhibition of the mycelium extracellular extract of the mushroom *P. djamor* against tested bacteria were more effective than that of ketoconazole and clotrimazole and comparable to streptomycin. The antibacterial activity of extracellular extract against five bacterial isolates is depicted in Table 3. The maximum inhibition zone and percentage inhibition was observed against *Ralstonia pickettii* followed by *Bacillus subtilis*. The minimum inhibition zone and percentage inhibition was observed against *Pseudomonas aeruginosa*.



Figure 2. Local people selling mushroom (in asterisk). (a) *Termitomyces heimii* (b) *Termitomyces heimii* and (c) *Lentinus squarrosulus* and *Pleurotus djamor*

Discussion

Lentinus sajor-caju, *L. squarrosulus*, *Pleurotus cornucopiae*, *P. djamor* and *P. ostreatus* were found to grow on dead wood of *Mangifera indica* as reported in earlier studies that sawdust of *M. indica* exhibited better growth of mushroom (Islam et al., 2009). About 80% of the mushroom sellers comprised of tribal population and the rest 20% belonged to non-tribal community. All tribals were found to sell only the wild edible mushrooms whereas the non-tribal used to sell the cultivated mushrooms procured from Mohanpur and Lake Chowmuhani, Tripura. Only 25% of the informants have shared the ethnomycological knowledge during the survey. The ethnic people could easily distinguish the poisonous from non-poisonous mushrooms as was evident from the absence of mushrooms poisoning. The collection of wild mushrooms by the ethnic tribes starts at 4.00 am and are described by different terms like Mikhumu in Kokborak; Mikhumu khapolok for mushrooms that grow on soil and are in association with termite nest; are Mikhumu aathikiri for mushrooms that grow only on soil and Buphangni mikhumu for

Table 3. Antibacterial activity of extracellular extract from submerged culture of *Pleurotus djamor*

Bacteria	Me		Kc		Ct		Sm	
	Inhibition zone (mm)	% Inhibition						
<i>Bacillus subtilis</i>	22.0±0.06	27.50	13.7±0.07	17.08	15.0±0.12	18.75	19.0±0.06	23.75
<i>Xanthomonas campestris</i>	18.3±0.09	22.92	15.0±0.10	18.75	12.3±0.03	15.42	12.7±0.03	15.83
<i>Pseudomonas aeruginosa</i>	14.0±0.00	17.50	10.3±0.09	12.92	11.7±0.03	14.58	18.3±0.03	22.92
<i>Ralstonia pickettii</i>	22.7±0.03	28.33	18.7±0.03	23.33	14.0±0.06	17.50	17.0±0.12	21.25
<i>Erwinia sp</i>	16.3±0.03	20.42	15.0±0.12	18.75	12.7±0.03	15.83	27.0±0.25	33.75

Me-Mycelium extract, Kc-Ketoconazole, Ct-Clotrimazole, Sm-Streptomycin

mushrooms that grow on trees. Mushrooms were sold together with snails, crab and other wild grown vegetables and fruits. Mushrooms were brought to the market in 3-5 kg polythene bags and were retail sold to the customers in polythene bags. 60% of the sellers consisted of women and the rest were men. The number of species sold by each seller in the market varied from 2 to 5 depending on the seasonal availability of the wild edible mushrooms and also on the ethnic tribes inhabiting a vicinity of the local market.

Generally, antimicrobial drugs have long been used for prophylactic and therapeutic purposes. This led to the search of new antimicrobial substances effective against pathogenic microorganisms resistant to conventional treatments. Among the new groups of organisms which have been increasingly explored in the recent years, mushrooms could be an alternative source for new antimicrobials (Alves *et al.*, 2012). The findings of the present investigation revealed better inhibitory action of the antibacterial activity of extracellular extract from submerged culture of *P. djamor* tested against selected strains of bacteria than antibiotic agents such as ketoconazole, clotrimazole and streptomycin. The extracts of *P. djamor* inhibited both Gram positive and negative bacteria suggesting broad-spectrum antimicrobial potential. The mycelium culture extracts of wild mushroom species are potentially rich source of antimicrobial agents (Rosa *et al.*, 2003; Tambekar *et al.*, 2006; Kalyoncu *et al.*, 2010) which is in accord to our study.

Conclusion

The mycophily among the ethnic people of Tripura is high considering the quantity of mushrooms sold in the markets of Tripura. Certain species of Termitomycetes and Tricholoma are favoured than other species but they are dependent on season for their availability. However, knowledge on cultivation of these wild edible mushrooms among the local ethnic people of Tripura should be given importance where commercialization can enhance mushroom consumption among the local tribal people and

economically uplift the society as a whole. The extracellular mycelia extract exhibit better activity comparatively than antibiotics studied. The future study should be directed towards the isolation of bioactive compounds from the extracellular extracts of mycelium of *P. djamor*.

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