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Antimicrobial Properties and Phytochemical Screening of Some Wild Macrofungi of Rani - Garbhanga Reserve Forest Area of Assam, India

Deka AC[1], Indrani Sarma[1*], Sneha Dey[2] and Sarma TC[2]

[1]*The Energy and Resources Institute, North Eastern Regional Centre. Guwahati, India*
[2]*Guwahati University, Department of Botany, Guwahati, India*

ABSTRACT

Natural products have potential of containing agents for various diseases. The increase in antibiotic-resistant bacteria has revived the interest in alternative antimicrobial substances from natural resources to control the pathogenic micro-organisms. The antimicrobial activity of few macrofungal species isolated from Rani-Garbhanga Reserve forest areas of Assam were used for screening of antimicrobial potential. Auricularia auricula-judae, Pleurotus ostreatus, Pleurotus tuber-regium, Pycnoporous sanguineus, Schizophyllum commune, Trametes elegans, Trametes versicolor and Tremella fuciformis are investigated against a panel of standard bacteria. The tested microorganisms includes Bacillus subtillis MTCC 736, Salmonella typhi MTCC 3216, Staphylococcus aureus MTCC 3160, Pseudomonas aeruginosa MTCC 7837 and Eschercia coli MTCC 40. Among the macrofungal species studied the ethanolic extracts of four macrofungi showed satisfactory results. Macrofungal extracts analyzed (water and ethanol) showed wider inhibition zones in disc-diffusion method for Trametes elegans and found to be most effective followed by Pleurotus tuber-regium, Auricularia auricular-judae. The phytochemical screening of the macro fungal extracts revealed the presence of secondary metabolites like glycosides, flavonoids, tannins, triterpenoids and saponins, etc. The present study reveals the biopharmaceutical potential of few indigenous macrofungal species of Rani-Garbhanga Reserve forest areas of Assam.

Keywords: Macrofungi, Disc diffusion assay, Bioactive compounds, *In vitro* antimicrobial activity

INTRODUCTION

The Indian sub-continent has favorable agro climatic conditions which promote natural growth of wide range of fungal species. The occurrence of macro fungi is of diverse nature and most of them are not well studied and documented. Northeastern region of India is one of the biodiversity hotspots with enormous macro fungal species. Macro fungi are cosmopolitan heterotrophic organisms grown in the wild and play an important role in nutrient cycling and carbon sequestration to maintain the forest health besides their medicinal importance and nutritional value. The macro-fungi can grow in soil or degrading plant residues as saprophytes, wood decaying and many live in symbiotic association with the roots of higher plant species. They play important role in nutrient recycling; growth and establishment of seedlings in forest floor and regarded as the indicator of Forest health and maturity [1]. It has been reported that several macrofungal species, such as *Trametes versiocolor*, serve as decomposers of organic persistent pollutants [2]. Macrofungi are also potential for bioremediation of industrial waste [3]. Till date only a fraction of the total fungal wealth of India has been subjected to scientific scrutiny. The peak values for species richness and season for the formation of fruit body of macrofungi is different for each ecological climate [4,5]. About 10,000 fungal genera are reported from the world, of which more than 2000 genera and 14,000 species are known from India [6]. Only a few macrofungi has been described so far and out of which a few numbers has been explored for the production of important pharmacological metabolites till now. Interestingly some of the most successful drugs and agrochemical fungicides have been developed from fungal secondary metabolites [7]. These include antibiotics (penicillins, cephalosporins and fusidic acid), antifungal agents (griseofulvin, strobilurins and echinocandins), cholesterol-lowering agents such as statin derivatives (mevinolin, lovastatin and simvastatin), and immunosuppressive drugs (cyclosporin) [8]. As the

percentage of economically valuable fungal metabolites are still small so there is scope for exploration of more myco-metabolites of medicinal properties. A numbers of macrofungal species have been used traditionally for centuries in Asia as potential nutritive and popular medicines to prevent or treat different diseases [9,10]. Due to unique climatic condition the North-East India and Assam in particular has a very rich and diverse species of macro fungi. Macro fungi that are naturally growing on different substrates such as leaf litter, decaying plant residues and decomposing logs of trees particularly during rainy season are reported earlier from various parts of India including NE region [11,12]. However, limited information's available on the medicinal uses of the macro fungi. In India, macro fungi are a non-wood forest produce and popular as food among the ethnic people of North east India. The ethnic tribes of Assam living near the forest areas hold a treasure house of traditional knowledge on nutritional and medicinal value of these bio-resources. Some macro fungal species are preferred by these ethnic tribes due to their deliciousness as food and sometime as alternative medicine. Macro fungi contain high quality protein and also serving as important source of vitamins, essential minerals for the poor ethnic population. Macrofungi are also reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. *Trametes versicolor* (anti-cancer) [13], *Ganoderma lucidum* (Anticancer) [14], *Schizophyllum commune* (antimicrobial and anticancer) [15], *Auricularia auricula-judae* (antitumor, anti-diabetic, antibacterial) [16], etc., are naming a few macrofungal species used in different traditional medicine. Some of the edible species like *Termitomyces eurrhizus*, *Lentinus conatus*, *Schizophyllum commune*, *Tricholoma giganteum* and *Pleurotus* are sold in the markets of Kohima district of Nagaland by the local people [12]. Kumar et al. described fifteen edible macro fungi along with their macronutrient content collected from different forest areas of Nagaland [17]. Similarly, twelve ethnomycologically important macrofungal species has been documented from Dhemaji district of Assam [18]. Four medicinal macrofungi from Nagaland along with their nutrient potentials has been reported [17]. However, few factors reported to be responsible for underutilization of the macrofungi like trade secret knowledge of local herbalist, seasonal nature, mycophobia, social stigma etc. Recently, edible macro fungi gaining popularity as food items for high protein with low calorific values and availability of essential minerals. Among the local communities, wild macro fungi may represent potential sources of antibacterial drugs, since in the early days, screening for antibiotics started with macro fungi and proved to be successful [19]. Even though there is tremendous progress in human medicine still bacterial, fungal and viral diseases threaten the public health in the developing countries due to development of drug resistance [20]. Human pathogenic microorganisms have developed multiple-drug resistance due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases. The rampant multi-drug resistance among human infectious pathogens has necessitated a continuous search for new and efficient alternative antimicrobial substance [21]. Some of the macrofungal species have been shown to be rich sources of natural antibiotics and accumulate a variety of chemicals with strong anti-oxidant properties [22]. These functional characteristics are mainly due to the presence of polysaccharides, dietary fiber and in particular chitin and beta glucans [23]. Secondary metabolites of most of the macrofungi shows antitumor, antibiotic, antiviral [24], anti-tumor, antithrombotic, immuno-modulating effects and anti-cancerous properties [10,25] and played vital role in various native medicines. Several authors have reported on the antimicrobial activities of different macro fungi and have associated these activities to the presence of varieties of secondary metabolites such as peptides, tannins, terpenoids, phenols and flavonoids [26,27]. Natural products have potential of containing therapeutic agents for various infectious diseases [28]. In last few years natural bio resources are exploited in different parts of the world and among them macro fungi could be an alternative source of new antimicrobials. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads but only a minute portion of the available diversity among fungal species has yet been explored for such purposes [29]. On search of new therapeutic alternatives researchers reported many kinds of therapeutic activities of wild macro fungal species including anti-carcinogenic, anti-inflammatory, immuno-suppressor and antibiotic activity etc. Though a large numbers of antimicrobial compounds have been isolated from macrofungi; however, compounds from microscopic fungi are dominated in the antibiotic market [30]. Moreover, many macro fungi are becoming extinct and facing threat of extinction in India because of habitat destruction, climate change, unorganized harvesting, deforestation, urbanization trends and population growth results the need for proper investigation, conservation, documentation of ethno-botanical importance and their scientific validation.

Therefore, it becomes quite necessary to explore, document, conserve and investigate medicinal properties of this natural wealth. The present study provides a database on medicinally important macrofungal species of Rani-Garbhanga Reserve forest, Assam, India along with their community utilization, which was not documented earlier.

MATERIALS AND METHODS

Sample collection, maintenance and preservation

Macrofungi sample collection

Fresh fruit bodies of ethnomedicaly important macrofungi were collected from rotten wood logs and leaf litters of forest cover areas of Garbhanga Reserve Forest, Assam following the standard technique [31,32] during rainy season

(June to September in 2015) and winter (October to December, 2015). Fruiting bodies were wrapped in the wax paper and putting into well marked polythene bags and taken to the laboratory for identification and antimicrobial assays. All macrofungi samples were identified on the basis of macro and microscopic characteristic following available literatures [33]. The taxonomy has been worked on the basis of macro and microscopic characteristic following available literatures [33,34]. The soft and hard textured specimens were preserved in 2% and 4% formaldehyde, respectively.

Collection of test organisms

Typed cultures like *Bacillus subtillis* MTCC 736, *Salmonella typhi* MTCC 3216, *Staphylococcus aureus* MTCC 3160, *Eschercia coli* MTCC 40 and *Pseudomonas aeruginosa* MTCC 7837 were collected from the IMTECH, Chandigarh. Bacterial isolates were subcultured on nutrient agar medium and incubated at 37°C for 24 h and subsequently subcultured and maintained for another 24-48 h at 37°C. The subcultured microorganisms were then stored at 4°C until needed.

Phytochemical screening

Phytochemical screening (qualitative) of macrofungal species extracts

A qualitative phytochemical analysis of the crude macrofungal extracts was carried out by following the standard protocols [35-37].

Test for alkaloids

Ethanolic extract of each Macro fungal species (0.5 g) were stirred with 5 ml of 1% aqueous hydrochloric acid (HCl) for two minutes on a steam water bath. After cooling the mixtures were filtered and few drops of Dragendorff's reagent were added. The change of the samples colour or turbidity was then recorded to draw inference.

Test for saponins

To screen the presence of saponins the persistent frothing test was carried out as described by Odebiyi and Sofowora [38]. Distilled water (30 ml) was added to 1 g of each of the macro fungal extracts, vigorousy shaken the mixture and heated on a steam water bath. The samples were then observed for the formation of froth to draw inference.

Test for phlobatannins

Each macrofungal extracts (0.2 g) were dissolved in 10ml of distilled water and filtered. The filtrates were boiled with 2% HCl solution and observed for deposition of red precipitate. The presence of red precipitate indicates the presence of phlobatannin.

Test for tannins

For screening for tannins the method of Trease and Evans [38] was adopted. Each sample (0.5 g) was dissolved in 5 ml of distilled water, followed by boiled gently and cooled. 1 ml of each solution was dispensed in a test tube and 3 drops of 0.1% ferric chloride solution were added. The change of colour observed for brownish green or blue black indicates the presence of tannins.

Test for terpenoids

For screening of terpenoids the Salkowski test was used. Macrofungal extracts (5 ml each) were mixed in 2 ml of chloroform and 3 ml concentrated sulphuric acid (H_2SO_4) were carefully added to form a layer. The change of colour observed for reddish brown coloration will confirms the presence of terpenoids [39].

Test for steroids

To screen the presence of steroids the macrofungal extract (0.5 g each) will be mixed with Acetic anhydride (2 ml) and filtered. Concentrated Sulphuric acid (2 ml) was added to the filtrate and observed for colour change from violet to blue or green, which indicates the presence of steroid.

Test for flavonoids

Diluted ammonia solution (5 ml) was added to portions of aqueous filtrate of each macro fungal extracts. This was then followed by the addition of a concentrated sulphuric acid. The solutions were observed for yellow coloration that disappears on standing to confirm the presence of flavonoids.

Test for anthraquinones

For the detection of anthraquinone Borntrager's test was used. The macro fungal extract (0.5 g) was shaken with 10ml

of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken and observed for the presences of pink red or violet color in the ammonia layer which indicates the presence of free anthraquinones.

Antimicrobial activity assay

Extraction procedure

All the collected fresh macro fungi samples were brush-cleaned of attached soil and humus and then air-dried in an oven at 40°C for 48 h. The cleaned fruiting bodies were then cut into bits, grounded by an electrical grinding machine and grounded samples were stored in an air-tight container for further use. The active substances of the grounded samples were extracted subsequently using ethanol and distilled water. The powdered macro fungal sample (100 g) was extracted individually by soaked in 2000 ml of 95% ethanol in different Erlenmeyer flask. The flasks was covered with aluminum foil and allowed to stand for 3 days in dark for extraction with occasional stirring. After 3 days the extracts were filtered through Whatman No. 1 filter paper (0.45 µm) and the filtrate was evaporated by rotary evaporator at 30°C with 90 rpm under reduced pressure. The obtained concentrated extracts were stored in dark at 4°C until further analysis. The residue was used to prepare different extract concentrations (50 $mg.mL^{-1}$, 100 $mg.mL^{-1}$, 200 $mg.mL^{-1}$ and 300 $mg.mL^{-1}$).

For water extractions (distilled water used) blending of slurry 100 g in 100 ml distilled water followed by mixing and kept static for 24-72 h. It was followed by centrifugation (15,000 rpm for 30 min, 2 to 3 times) for clarification.

Preparation of inocula

Bacillus subtillis MTCC 736, *Salmonella typhi* MTCC 3216, *Staphylococcus aureus* MTCC 3160, *Eschercia coli* MTCC 40 and *Pseudomonas aeruginosa* MTCC 7837 were used in the study. All bacterial strains were collected from IMTECH, Chandigarh and cultured in laboratory. Inocula were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standards having approximately 10^8 cfu/ml for bacteria. Mueller-Hinton Agar (Merck) medium and Nutrient broth media are used for the bacterial culture.

Determination of antimicrobial activity of macrofungal extracts

The antibacterial activity assay was based on the disc diffusion assay using Bacterial cell suspension grown at 37°C in LB media. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient Broth (NB) and were incubated without agitation for 24 h at 37°C [40]. The exponentially growing bacteria (OD 600=0.5, 10 12 cfu/mL) were mixed with melted warm LB agar and pour in to the Petri dishes. The ethanol extracts of the macrofungi were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 10 mg/mL, and filter sterilized through 0.45 µm membrane filter. One hundred microliters of the extract of each isolate of mushrooms was loaded into the pre sterilized paper disc and allow to air dry in sterilized condition to get rid of any residual solvent which might interfere with the results. After air drying these sterile paper discs were placed on the inoculated agar plates of different microorganisms. Similarly also sterile paper disc prepared for distilled water extracts and placed in the agar plates. Disc with distilled water treated as control and vancomycin discs were used as positive controls. Sterile paper discs with the extracts, vancomycin discs and control were placed on the agar. Discs were firmly applied to the surface of the plate which had an even contact with the agar. The Plates were incubated at 37°C for 24 h. After the incubation period the zone of inhibition was measured. In this experiment vancomycin was used as positive control and DMSO was used as negative control for the test microorganisms.

Determination of minimum inhibitory concentration

The MIC was determined by establishing a visible growth of microorganisms.

The MIC was defined as the lowest concentration of the compounds to inhibit the growth of tested microorganisms. The MIC values were studied for the microbial strains, being sensitive to the extracts in the Paper disc diffusion method. From the stock solutions of the extracts initially prepared a concentration of 10 mg/mL of extract was added into the first wells. DMSO solution (30%) was used as a negative control. Then, dilutions were made so as to decrease the concentration of extract by 1 mg at each dilution. Positive and negative controls and incubation conditions were the same as in the zone of inhibition assay. Visible growth (turbidity) in the dilution tubes was the criterion to determine MIC values for the tested microorganisms at the given concentration of each extract. The extract in this study was tested in triplicate against each test organism.

Ethanol extracts of *Auricularia auricula*-judae (Bull.) J. Schröt, *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm., *Pleurotus tuber*-regium (Rumph. ex Fr.), *Pycnoporous sanguineus* (L.) Murril., *Schizophyllum commune* Fries, *Trametes elegans* (Spreng.) Fr. and *Tremella fuciformis* Berk were studied for determination of MIC. The tests were performed in triplicates.

Controls

All extraction solvents with DMSO solution and empty sterile discs were used as negative controls.

RESULTS

The mushroom gatherers have good knowledge about the morphological characters including structures of each macrofungal species growing in their locality. The traditional health practitioners also able to identify medicinally important macro fungal species. and able to differentiate the edible macrofungi from the poisonous ones. Based on the knowledge of local inhabitant few medicinally important macrofungal species like *Auricularia auricular*-judae, *Pleurotus ostreatus. Pleurotus tuber*-regium, *Pycnoporus sanguineus*, *Trametes elegans*, *Trametes versicorol*, *Schizophyllum commune* and *Tramella* sp. are investigated.

Phyto-chemical screening

The wild grown macro-fungal species are collected by the villagers living near to the forest areas and some of them even sell it in the local market. The community utilized macrofungal species of medicinal importance of Rani-Garbhanga area were considered for phytochemical screening. Results from the phytochemical analysis revealed the presence of saponins, tannins, steroids, terpenoids, flavonoids in all the macro fungal species extracts, while anthraquinones, alkaloids and phlobatannins were absent in most of the species tested. The qualitative values of the various phytochemicals present in different macro fungal species were presented in Table 1. From the qualitative screening it was observed that Saponin, Tannin, Steriod, Terpenoid, Flavo noids etc were observed in the entire macrofungal sample tested whereas there was no response for Alkaloid, Anthra quinine and Phlobatannin (Table 1).

Antimicrobial activity study

In this study the antimicrobial activity of *Auricularia auricular*- judae, *Pleurotus ostreatus*. *Pleurotus tuber*-regium, *Pycnoporus sanguineus*, *Trametes elegans*, *Trametes versicorol*, *Schizophyllum commune* and *Tramella* sp. are investigated against *Pseudomonas aeruginosa* (MTCC No. 7837), *Salmonella typhi* (MTCC No. 3216), *Staphylococcus aureus* (MTCC No. 3160), *Bacillus subtilis* (MTCC No.736) and *Escherichia coli* MTCC 40. The fruiting bodies of tested macrofungal species are shown in Figure 1.

All the mushrooms used in this study were found to exhibit various degrees of antimicrobial effects against the tested microorganisms. It is observed that both ethanolic and crude (distilled water) extracts had a degree of antibacterial

Table 1: Qualitative phytochemical screening of community utilized macrofungal extracts

Macro fungi species	Community utility	Phytochemicals response (+/--)							
		Saponin	Tannin	Steriod	Alkaloid	Terpenoid	Flavonoids	Anthraquinone	Phlobatannin
Trametes elegans	Used in cut injury	++	+++	++	--	++	++	--	--
Pleurotus ostreatus	Used for health care	+++	+++	++	--	++	++	--	--
Pleurotus tuber-regium	Used for stomach pain	++	++	++	--	++	++	--	--
Auricularia auricular-judae	Used as food, antimicrobial	++	+++	++	--	++	++	--	--
Pycnoporus sanguineus	Used for healthcare	++	+++	++	--	++	++	--	--
Schizophyllum commune	Used to relief from headache	++	++	++	--	++	++	--	--
Trametes versicolor	Used to increase immunity	++	+++	++	--	++	++	--	--
Tremella fuciformis Berk	Used in stomach pain	++	++	++	--	++	++	--	--

Table 2: Antimicrobial assay of few macrofungal species collected from Rani-Garbhanga Reserve Forest area of Assam

S. No.	Macro fungal sample	Extract	*Bacillus subtillis* MTCC 736	*Salmonella typhi* MTCC 3216	*Staphylococcus aureus* MTCC 3160	*Eschercia coli* MTCC 40	*Pseudomonas aeruginosa* MTCC 7837
1	*Auricularia auricular*-judae	Ethanol	7.92	3.14	1.76	1.69	4.74
		Water	4.25	2.20	1.42	2.55	1.46
2	*Pleurotus ostreatus*	Ethanol	3.45	ND	ND	3.39	3.55
		Water	4.66	4.51	ND	3.80	6.64
3	*Pleurotus tuber*-regium	Ethanol	8.29	3.18	6.06	5.42	7.10
		Water	4.06	ND	2.91	6.14	6.64
4	*Pycnoporus sanguineus*	Ethanol	4.42	1.18	ND	3.21	6.54
		Water	3.22	ND	ND	ND	ND
5	*Trametes elegans*	Ethanol	9.66	4.11	2.20	11.74	5.27
		Water	5.09	3.30	1.71	6.33	2.03
6	*Trametes versicorol*	Ethanol	1.19	3.33	1.27	ND	3.93
		Water	ND	1.33	ND	ND	ND
7	*Schizophyllum commune*	Ethanol	2.70	5.32	2.39	7.33	5.18
		Water	2.28	ND	ND	4.36	4.73
8	*Tramella* sp.	Ethanol	ND	5.53	2.83	ND	ND
		Water	ND	3.60	2.94	2.55	1.42

ND: Not Determined

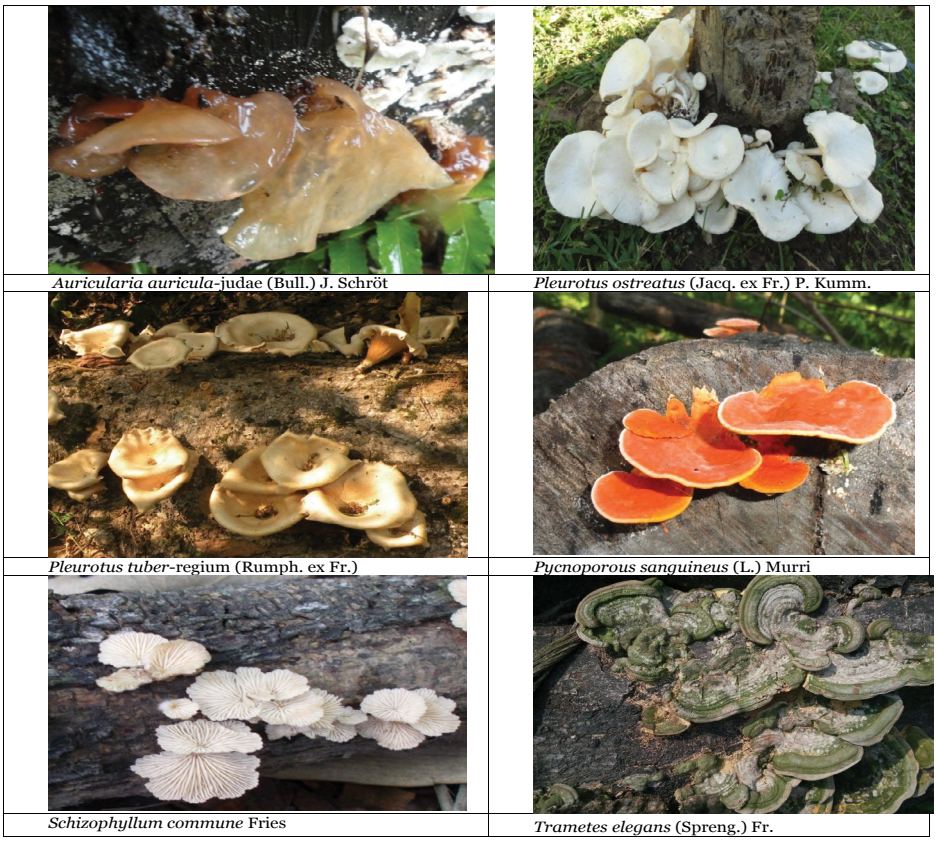
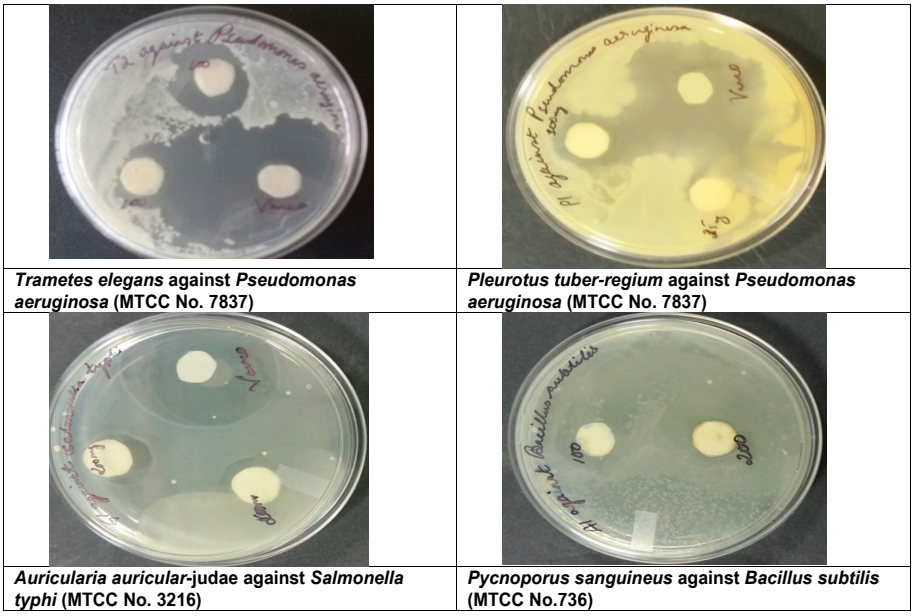


Figure 1: Fruiting body of macrofungal species of ethnomedicinal values collected from natural habitat (Rani-Garbhanga Reserve Forest, Kamrup, Assam)

activity against the selected bacterial species. The zone of inhibition exhibited more than 15 mm was considered as highly active extracts against the microbial pathogen tested. Among the macrofungal species studied the ethanolic extracts of four macrofungi showed significant microbial inhibition. The commercial antibiotics used, vancomycin (5 µg) gave the highest antibacterial activity. The activities of the commercial drugs when compared to the macrofungal extracts were found to be slightly higher however in case of *Trametes elegans* and *Pleurotus tuber*-regium the results

Table 3: Minimum inhibitory concentration (MIC mg/ml) of ethanol extracts of macrofungal fruit body extracts and commercial antibiotic against test organisms

Test organism	Macrofungal extract							
	***Auricularia auricular*-judae**	***Pleurotus ostreatus***	***Pleurotus tuber*-regium**	***Pycnoporus sanguineus***	***Trametes elegans***	***Trametes versicorol***	***Schizophyllum commune***	***Tremella fuciformis* Berk**
Bacillus subtillis MTCC 736	12.5	10.0	12.5	12.5	25	ND	10.0	12.5
Salmonella typhi MTCC 3216	12.5	12.5	12.5	12.5	25	12.5	12.5	12.5
Staphylococcus aureus MTCC 3160	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Eschercia coli MTCC 40	50	25	50	25	50	25	25	25
Pseudomonas aeruginosa MTCC 7837	50	50	50	12.5	50	ND	ND	12.5



***Trametes elegans* against *Pseudomonas aeruginosa* (MTCC No. 7837)** | ***Pleurotus tuber-regium* against *Pseudomonas aeruginosa* (MTCC No. 7837)**

***Auricularia auricular*-judae against *Salmonella typhi* (MTCC No. 3216)** | ***Pycnoporus sanguineus* against *Bacillus subtilis* (MTCC No.736)**

Figure 2: The minimum inhibitory concentration (MIC) of the macrofungal extracts ranged from 10.0 to 50 mg/ml, while with the referenced bacteria exhibiting lesser MIC values

for microbial inhibition activity was found to be equivalent to the commercial drug (Table 2). Macrofungal extracts analyzed (water and ethanol) showed wider inhibition zones in disc-diffusion method for *Trametes elegans* and found to be most effective against the tested bacterial pathogens. It was followed by *Pleurotus tuber*-regium and *Auricularia auricular*-judae. Moderate response was also observed for *Pycnoporus sanguineus*, *Schizophyllum commune* and *Trametes versicolor*. It was also observed that *Trametes elegans* and *Pleurotus tuber*-regium were also found to be very effective against most of the bacterial species tested in comparison to positive controls (Table 3 and Figure 2).

DISCUSSION AND CONCLUSION

Mushrooms require antibacterial and antifungal compounds in order to survive in their natural habitat [40,41]. These attributes might make them rich sources of natural antibiotics. The cultural and biochemical characteristics of Macrofungal species obtained in this study are typical of the species (Tables 1 and 2). Eight numbers of macrofungal species of ethnomedicinal importance viz. *Auricularia auricular*- judae, *Pleurotus ostreatus*. *Pleurotus tuber*- regium, *Pycnoporus sanguineus*, *Trametes elegans*, *Trametes versicorol*, *Schizophyllum commune* and *Tramella* sp. were collected from Rani-Garbhanga Forest during rainy season. The phytochemical properties and the antimicrobial potential of extracts of these macrofungal species indigenous to Assam were assessed in this study. The present study shows that macrofungal extract are antagonistic against bacterial pathogens which is in accordance with the

previous reports [42]. Results from the extraction process showed ethanol giving a better response for phytochemical screening and antimicrobial assays. This is mainly due to the ability of the solvent to dissolve endogenous compounds [43]. It has been reported that polar solvents to be more effective in extracting organic and inorganic materials from biological sources [44]. Similar results were also reported by other workers [40]. Moreover, some other factors, such as the chemical nature of the compounds, the extraction method employed, the extraction solvent, the extraction time and conditions such as temperature and pH of solvent, and the presence of interfering substances, can influence the extraction process [45]. The extracts of macro fungi species used in the present study displayed varying antimicrobial activities. Depending upon the test organisms, nature of environment and media in which the test organism grows, solvent used for extraction, genetic makeup of the macrofungi and differences in physical and biochemical nature of the antimicrobial components available in the extracts [46,47]. Results from the qualitative phytochemical analysis revealed the presence of saponins, tannins, steroids, terpenoids, flavonoids and cardiac glycosides in all the macrofungal species extracts, while alkaloids, anthraquinones and phlobatannins were absent. The minor variation in the biologically active phytochemicals results in changes in biochemical properties. The variation of biochemical properties and antimicrobial activities among the studied species might be due to species which also influence by their habitat [48]. The variation of biologically active phytochemicals serves as a defense mechanism against predation by many microorganisms, insects and other herbivores [49]. The differences in the antimicrobial activities of different species of mushrooms have been mainly attributed to the differences in the antimicrobial components found in them [50]. This suggests that the macrofungi can be used in the treatment of infectious diseases. Antimicrobial activity of tannin extracted from *Rhizophora apiculata* bark has been reported [51]. The secondary metabolites of macrofungi influence on the antimicrobial properties of the species. Similar microbial inhibition due to presence of saponins has been reported earlier. The antimicrobial activity of the studied species might be due to presence of steroids as reported by other worker. Antimicrobial activity of *Trametes elegans* has also been reported by earlier worker [50].

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REFERENCES

[1] Kumar R, Bisht NS, Mishra G, Kalita K, Bezbaroa NR. Micro and macrofungal diversity in Langol herbal garden Manipur, India. Int J Curr Life Sci, 2015, 1: 24-34.

[2] Tran NH, Urase T, Ngo HH, Hu J, Ong SL. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Biores Tech, 2013.

[3] Kalac P, SvobodaL, Havilkova B. Content of cadmium and mercury in edible mushrooms. J Appl Biomed, 2004, 2: 15-20.

[4] Arora D. All that the rain promises and more. A hip pocket guide to western mushroom. Ten Speed Press, New York, 1991.

[5] Baptista P, Martins A, Tavares RM, Lino-Neto T. Diversity and fruiting pattern of macrofungi associated with chestnut (Castanea sativa) in the trás-os montes region (northeast Portugal). Fungal Ecol, 2010, 3: 9-19.

[6] Maini H, Shukla A. Freshwater fungal richness, their assessment and impact on human welfare: A review. *Int J Sci Res*, **2016,** 2319-7064.

[7] De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, et al. Bioactive metabolites from macrofungi: Ethnopharmacology, biological activities and chemistry. *Fungal Divers*, **2013**, 62:1-40.

[8] Kozlovskii AG, Zhelifonova VP, Antipova TV. Fungi of the genus Penicillium as producers of physiologically active compounds (review). *Appl Biochem Microb,* **2013**, 49: 1-10.

[9] Cheung LM, Cheung PCK. Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chem*, **2005**, 89: 403-409.

[10] De Silva DD, Rapior S, Fons F, Bahkali AH, Hyde KD. Medicinal mushrooms in supportive cancer therapies: An approach to anti-cancer effects and putative mechanisms of action. *Fungal Divers,* **2012**, 55: 1-35.

[11] Tapwali A, Kumar R, Pandey. Diversity and frequency of macrofungi associated with wet ever green tropical forest in Assam, India. *Biodiversitas*, **2013,** 14: 73-78.

[12] Ao T, Seb J, Ajungla T, Deb CR. Diversity of wild mushrooms in Nagaland, India. *Open Journal of Forestry*, **2016**, 6: 404-419.

[13] Ramberg JE, Nelson ED, Sinnott RA. Immunomodulatory dietary polysaccharides: A systematic review of the literature. *Nutrition J*, **2010**, 9: 1-22.

[14] Wachtel-Galor S, Szeto YT, Tomlinson B, Benzie IF. *Ganoderma lucidum* ('ling zhi'): Acute and short term biomarker response to supplementation. *Int J Food Sci Nutr,* **2004**, 55: 75-83.

[15] Wasser SP. Current findings, future trends and unsolved problems in studies of medicinal mushrooms. *Appl Microbiol Biotechnol*, **2011**, 89: 1323-1332.

[16] Gurusamy R, Arthe R. Effect of medicinal mushroom, *Auricularia auriculajudae*, polysaccharides against EAC cell lines. *Res J Biotech*, **2012**, 7: 14-17.

[17] Kumar R, Tapwal A, Pandey S, Borah RK, Borah D, et al. Macro-fungal diversity and nutrient content of some edible mushrooms of Nagaland, India. *Nusantara Biosci*, **2013**, 5: 1-7.

[18] Gogoi Y, Sarma TC. An ethnomycological survey in some areas of Dhemaji district (Assam). Proceedings of International Conference on Anthropogenic Impact on Environment & Conservation Strategy, Ranchi, **2012**.

[19] Opige M, Kateyo E, Kabasa JD, Olila D. Antibacterial activity of extracts of selected indigenous edible and medical mushrooms of eastern Uganda. *Int J Trop Med*, **2006**, 1: 111-116.

[20] Okeke IN, Laxminarayan R, Bhutta ZA. Antimicrobial resistance in developing countries. Part 1: Recent trends and current status. *Lancet Infect Dis*, **2005**, 5: 481-493.

[21] Lallawmsanga, Passari AK, Mishra VK, Leo VV, Singh BP, et al. *Ganoderma lucidum* ('Lingzhi'), a Chinese medicinal mushroom: Biomarker responses in a controlled human supplementation study. *Br J Nutr*, **2004**, 91: 263-269.

[22] Keleş A, Koca İ, Gençcelep H. Antioxidant properties of wild edible mushrooms. *J Food Process Technol*, **2011**, 2: 130.

[23] Reshetnikov SV, Wasser SP, Tan KK. Higher basidiomycetes as a source of antitumor and immunostimulating polysaccharides. *Int J Med Mushr*, **2001**, 3: 361-394.

[24] Bandara AR, Rapiord S, Bhat DJ, Kakumyana P, Chamyuang S, et al. *Polyporus umbellatus*, an edible-medicinal cultivated mushroom with multiple developed health-care products as food, medicine and cosmetics: A review. *Cryptogamie Mycologie,* **2015**, 36: 3-42.

[25] Mau LL, Lim HC, Chen CC. Antioxidant properties of several medicinal mushrooms. *J Agric Food Chem*, **2002**, 50: 6072.

[26] Alves MJ, Ferreira IC, Froufe HJ, Abreu RM, Martins A, et al. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *J Appl Microbiol*, **2013,** 115: 346-357.

[27] Lavanya J, Subhashini S. Therapeutic proteins and peptides from edible and medicinal mushrooms-Review. *ESJ*, **2013**, 9: 1857-7881.

[28] Clardy J, Walsh C. Lessons from natural molecules. *Nature*, **2004**, 432: 829-837.

[29] Cos P, Vlietinck AJ, Vanden Berghe D, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. *J Ethnopharmacol*, **2006,** 106: 290-302.

[30] Wong KH, Vikineswary S, Noorlidah A, Umah RK, Murali N. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of *Hericium erinaceus* (Bull: Fr.) Pers. *Ext Food Tech Biot*, **2009**, 47: 47-55.

[31] Metzler S, Metzler V. Texas mushrooms, University of Texas Press, **1992**, 349.

[32] Mueller GM, Bills GF. Introduction. In: Biodiversity of Fungi Inventory and Monitoring Method, Mueller GM, Bills GF, Foster MS (edtrs), Elsevier Academic Press, San Diego, **2004**, 777.

[33] Purakasthya RP, Chandra A. Manual of Indian edible mushrooms. Today and Tomorrow's Publication, New Delhi, **1985**, 265-270.

[34] Alexopoulos CJ, Mims CW, Blackwell. Introductory Mycology. John Wiley and Sons Inc., New York, **1996**.

[35] Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants. *Lloydia*, **1978**, 41: 234-246.

[36] Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. Springer Pvt. Ltd., New Delhi, **2005**.

[37] Awala SI, Oyetayo VO. The phytochemical and antimicrobial properties of the extracts obtained from *Trametes elegans* collected from Osengere in Ibadan, Nigeria. *Jordan J Biol Sci,* **2015**, 289-299.

[38] Trease GE, Evans WC. Pharmacognosy. ELBS/Bailliere Tindall, London, **1989**, 345-346.

[39] Lukáš H, Matúš K, Jana H, Martin R, Jana P, et al. Antimicrobial activity of crude extracts from some medicinal mushrooms. *J Microb Biotech Food Sci*M **2016**, 5: 60-63.

[40] Lindequist U, Niedermeyer THJ, Julich WD. The pharmacological potential of mushrooms-Review. *J Evid Based Complementary Altern Med*, **2005**, 2: 285-299.

[41] Thillaimaharan KA, Sharmila K, Thangaraju T, Karthick M, Kalaiselvam M. Studies on antimicrobial and antioxidant properties of oyster mushroom *Pleurotus florida*. *Int J Pharma Sci Res*. **2016**, 4: 1540-1545.

[42] Anokwuru CP, Esiaba I, Ajibaye O, Adesuyi AO. Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. *J Med Plants Res*, **2011**, 5: 557-566.

[43] Campos AR, Albuquerque FAA, Rao VSN, Maciel MAM, Pinto AC. Investigations on the antinociceptive activity of crude extracts from *Croton cajucara* leaves in mice. *Fitoterapia,* **2002**, 73: 116-120.

[44] Brahmi F, Madani K, Dahmoune F, Rahmani T, Bousbaa K, et al. Optimisation of solvent extraction of antioxidants (phenolic compounds) from Algerian mint (*Mentha spicata* L.). *Phcog Commn,* **2012**, 2: 72-86.

[45] Iwalokun BA, Usen UA, Otunba AA, Olukoya DK. Comparative phtyochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *Afr J Biotechnol,* **2007**, 6: 1732-1739.

[46] Ramesh C, Manohar GP. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India. *Pharmacognosy Res*, **2010**, 2: 107-112.

[47] Celik GY, Onbasli D, Altinsoy B, Alli H. *In vitro* antimicrobial and antioxidant properties of *Ganoderma lucidum* extracts grown in Turkey. *Eur J Med Plants*, **2014**, 709-722.

[48] Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran*, J Biol Sci*, **2004**, 4: 405-412.

[49] Lim SH, Darah I, Jain K. Antimicrobial activities of tannins extracted from *Rhizophora apiculata* barks. *J Trop Forens Sci*, **2006**, 18: 59-65.

[50] Sahelian R. Saponin in plants benefit and side effects, glycosides and extraction. **2014**.

[51] Kabuyi MK, KapepulaPM, Kabengele JK, Moni B, Makengo G, et al. Selenium content and antioxidant potential of some edible wild mushrooms from Bandundu Area, DR Congo. *Nat Resour*, **2017**, 8: 103-113.