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Biological activities of *Schizophyllum commune* Fr. : A wild edible mushroom of Tripura, North East India

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Schizophyllum commune Fr., a wild edible mushroom was collected from forest bed of Tripura, North-East India. The proximate compositions along with antimicrobial and antioxidant activity of *S. commune* were evaluated. Apart from moisture content, the carbohydrate content was highest which is followed by, fibre, protein, fat, lipid and ash. The optimal temperature, pH and incubation time for the mycelial growth of *S. commune* were 25ºC, pH 6.0 and 21 days respectively. The best carbon and nitrogen sources for optimal mycelial growth were starch and yeast extract respectively. The highest inhibition of growth recorded against *Bacillus subtilis* (Gram positive) and *Xanthomonas campestris* (Gram negative). The methanolic extract of *S. commune* has potential antioxidant activity, although it contained 2.0 mg/g phenol. *S. commune* could be a good source of nutraceuticals.

Key words: Antimicrobial, antioxidant, mycelia growth, nutraceutical, proximate composition, *Schizophyllum commune*

INTRODUCTION

Tripura is the third smallest state of India. It is located in the south-west extreme corner of the north-eastern region, between latitude 22º57' and 24º33' N and longitude 91º10' and 92º20' E. *S. commune* is a species of Basidiomycetes belonging to the Schizophyllaceae family of order Agaricales. Mushrooms are considered a source of physiologically beneficial components with possible medicinal applications (Khan and Tania, 2012). *Schizophyllum commune* is commonly known as split gill and normally associated with white rot decay of wood. In the recent years, the antioxidant and antimicrobial activity received much attention because of the increasing interest in human health and have been studied in vitro and in vivo by many researchers (Singdevsachan *et al.* 2013). The developing countries like India with rich biodiversity of mushrooms are a boon for progress in the field of food and medicine and several nutriceuticals (Ajith and Janardhanan, 2007). Evaluation of the optimum conditions of submerged culture for production of mycelial biomass along with proximate composition were analyzed. The antimicrobial and antioxidant properties from dried mushroom of *S. commune* were assessed.

MATERIALS AND METHODS

Sample

The specimen was collected from forest bed of Mandwi (N 23°19.903' E 091°29.192'), West Tripura, North-East India. The mushroom sample was identified by comparing the descriptions with

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the work of Pegler (1977), and Purkayastha and Chandra (1985). Pure mycelial culture were obtained using tissue from junction point of stipe and pileus of fresh fruit body and maintained on Potato Dextrose Agar (PDA) medium. The culture collection number was MCCT 38. The mushroom samples were dried in hot air oven within the range of 45ºC to 55ºC for 24 hr and preserved in polyethylene bag by adding 1, 4-dichlorobenzene as disinfectant for further analysis.

Toxicity test

Toxicity test of wild mushrooms was carried out by the standard method (Svrcek, 1998). The color change was the primary indicator for presence or absence of toxins.
For the conformation of presence or absence of toxins (amatoxins and phallotoxins) in the test fungi, paper chromatographic method was used (Block *et al.* 1955).

Determination of moisture, crude fat and crude fibre content

Moisture content was determined by the method of AOAC (1960 and 1990).

Ash content

Ash content was determined by using the method of Raghuramulu *et al.* (2003).

Protein content

The total soluble protein was estimated by slightly modified method of Lowry *et al.* (1953). The standard curve for protein was prepared by using Bovine Serum Albumin (BSA).

Total lipid

Total lipid was determined by slight modified method of Folch *et al.* (1957).

Carbohydrate content

Total carbohydrate content was estimated by the method of Hedge and Hofreiter (1962). Glucose was used as standard.

Inoculum and culture medium preparation

A small portion of the actively growing mycelium from an agar slant of the test fungus was aseptically transferred to a sterile 250 ml conical flask containing 50 ml of Basal Synthetic Liquid (BSL) medium and was incubated on a shaking incubator (120 rpm) at 25°C (±5°C) for 7 days in complete darkness. After 7 days, the mycelia mat was aseptically fragmented into small pieces with the help of a waring blender. The fragmented mycelium washed several times with distilled water to remove any trace of adhering medium and suspended in a phosphate buffer medium (pH-5.5) for 24 hr to overcome the shock encountered during blending. An aliquot of 1 ml of mycelial cell suspension was used as the inoculum.
Fungal biomass productions were determined by the mycelial dry weight method. Each experiment was done in triplicates.

Effect of incubation time, temperature and pH

The effect of incubation time, temperature and pH on *S. commune* for biomass production was determined by using Basal Synthetic Liquid (BSL) medium. 40 ml of the basal medium was dispensed in each 100 ml conical flask. Each flask was inoculated aseptically with an aliquot of 1 ml of the mycelia which was previously prepared and incubated at 15°C, 20°C, 25°C, 30°C, and 35 °C separately for 7, 14, 21 28 and 35 days in each temperature by keeping in dark condition. The initial pH value of BSL medium was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 in each separate set of experiment. Sterilized pH adjusted medium was inoculated and incubated at 25°C on a shaker incubator (120 rpm) for 21 days and biomass was measured.

Uses of different carbon sources and nitrogen sources

The BSL media with different carbon and nitrogen sources were used. Seven carbon sources namely glucose, fructose, sucrose, maltose, starch, mannitol and xylose were evaluated separately to the fermentation medium as a sole carbon source using 20 g/l. The basal medium that lacks any carbon compound (0%) served as the control. Different sources of nitrogen (beef extract, peptone, yeast extract, urea, arginine, glycine, ammonium sulphate and ammonium nitrate) were used separately as sole nitrogen source using 5 g/l. The inoculated culture flasks were incubated for 21 days at 25°C on a shaker incubator (120 rpm) and the initial pH value was 6.0.

Extract preparation from dried mushroom of S. commune

Preparation of methanolic extracts of mushroom was done based on slightly modified method of Mau *et al.* (2004). Extract of *S. commune* was used for analysis of antimicrobial and antioxidant activity.

Antibacterial activity
Test microorganisms

Antimicrobial activity was tested on gram positive bacteria like *Staphylococcus aureus*(MTCC 96) and *Bacillus subtilis* (MTCC 619) ; gram negative bacteria like *Xanthomonas campestris* (MTCC 2286), *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa*(MTCC 424). Test bacterial strains were procured from IMTECH Chandigarh, India.

Antibacterial activity

The antibacterial activities were evaluated by the disc diffusion method (Collins and Lyne, 1987). To evaluate the antibacterial activity, bacteria were grown in liquid LB (Luria Bertani) medium for 24 h and after this growth period 100 ìl bacterial cultures spread on Petri dishes containing solid LB medium. The discs (4 mm diameter) were then impregnated with 100 µl of mushroom extract and then placed on solid LB medium.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

Antioxidant activity was determined by slightly modified method of Mau *et al.* (2004). 4 ml dried mushroom extract (0.25- 16 mg/ml) in methanol was mixed with 1 ml of a methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of 0.2 mM concentration (Sigma). The mixture was shaken vigorously and left to stand for 30 min in dark and the absorbance was measured at 517 nm against a blank. Percentage of inhibition= [(A Blank – A Sample) × A Blank] ×100. Where, A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound.

Fungal phenol estimation

Total phenol was determined according to the method of Swain and Hillis, (1959).

RESULTS AND DISCUSSION

Toxicity test

Toxicity test of *S. commune* showed negative result which means it contained no toxins. No blue or violet coloure spot were found to appear in the chromatographic strips. These observations indicated absence of any amanitin and phalloidin toxins in the test fungus.

Proximate Composition

The moisture, crude protein, crude fat, ash, total lipid, crude fiber and total carbohydrate content of *S. commune* were depicted in Table 1. Moisture content was highest and total lipid content was lowest. The nutritional components were crude fat (9.0%), crude fibre (30.0%), ash (3.5%), protein (15.55%), lipid (0.4%) and total carbohydrates (42.0%).

Submerged mycelial optimum growth requirement

In this study incubation time, temperature, pH, carbon and nitrogen sources were trialed for optimum biomass production. The results depicted that maximum mycelial biomass (15.00 g/l) was obtained at 25°C and above this temperature, the mycelial biomass production was decreased (Table 2). The best incubation time for *S. commune* was 21 days in which maximum mycelial biomass (15.50 g/l) produced (Table 3). The optimum pH for growth of *S. commune* was found to be 6.0 exhibiting highest vegetative growth of 14.50 g/l (Table 4). It was observed that separate supplementation of each of the various carbon sources affected mycelial growth of *S. commune* significantly. It was recorded that all the carbon sources showed better biomass production in comparison with control. Out of seven carbon sources, starch (10.50 g/l) and maltose (10.00 g/l) produced maximum mycelial biomass. Mannitol (2.50 g/l) as carbon source showed the lowest mycelial biomass production (Table 5). Effect of organic and inorganic nitrogen sources on growth of *S. commune* varied differently. The best biomass yield (8.65 g/l) was found in Yeast extract closely followed by ammonium sulphate (6.65 g/l) and lowest biomass yield was in ammonium nitrate (0.31 g/l), depicted in Table 6.

Table 1 : Proximate composition (%) of the wild mushroom *S. commune* on dry weight basis

Sample name	Moisture content	Crude fat content	Crude fiber content	Ash content	Crude protein	Total lipid	Total carbohydrates
S. commune (MCCT 38)	87.76	9.0	30.0	3.5	15.55	0.4	42.0

Table 2 : Effect of temperature for the mycelial growth of *S. commune* in the BSL medium

Mushroom sample	Different temperatures (g/l)				
	15°C	20°C	25°C	30°C	35°C
S. commune	4.00±0.01	10.75±0.06	15.00±0.02	7.00±0.01	6.50±0.03

Table 3 : Effect of incubation time for the mycelial growth of *S. commune* in the BSL medium

Mushroom sample	Different incubation time(g/l)				
	7 days	14 days	21 days	28 days	35 days
S. commune	0.75±0.01	7.75±0.06	15.50±0.00	15.00±0.00	14.00±0.01

Table 4 : Effect of pH for the mycelial growth of *S. commune* in the BSL medium

Mushroom sample	Different pH(g/l)						
	4.5	5.0	5.5	6.0	6.5	7.0	7.5
S. commune	4.50±0.00	7.00±0.03	6.25±0.02	14.50±0.01	9.25±0.00	13.00±0.01	13.00±0.01

Table 5 : Effect of different carbon sources of *S. commune* in the BSL medium

Mushroom sample	Different carbon sources(g/l)							
	Control	Dextrose	Fructose	Mannitol	Starch	Xylose	Maltose	Sucrose
S. commune	0.37±0.01	3.67±0.02	3.75±0.03	2.50±0.01	10.50±0.00	4.25±0.02	10.00±0.02	9.50±0.01

Table 6 : Effect of different nitrogen sources of *S. commune* in the BSL medium

Mushroom sample	Different nitrogen sources (g/l)								
	Control	Ammonium nitrate	Peptone	Urea	Yeast extract	Arginine	Glycine	Beef extract	Ammonium sulphate
S.commune	0.11±0.04	0.31±0.02	4.97±0.02	2.00±0.00	8.65±0.02	0.75±0.04	0.45±0.03	1.85±0.03	6.65±0.02

Table 7 : Antibacterial activity of methanolic extract of *S. commune*

Mushroom sample	Bacterial strains				
	Staphylococcus aureus	*Xanthomonas campestris*	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
S. commune MCCT 38	+	++	+	++	-

+ = produced inhibition zone. - = did not produce inhibition. ++= produced highest inhibition zone

Antibacterial activity

Antibacterial activities of methanolic extract from dried mushroom *S. commune* are shown below in Table. 7. The higher inhibition of growth recorded against *Bacillus subtilis* and*Xanthomonas campestris* (Fig. 2, A and B) in comparison with those of *Staphylococcus aureus* and *Escherichia coli* (Fig. 2, C and D). Methanolic extract of *S. commune* showed no activity against *Pseudomonas aeruginosa* bacteria.

Antioxidant activity

1,1-diphenly-2-picrylhydrazyl (DPPH) is a stable free radical that shows a characteristic absorbance at 517 nm, which decreases significantly when exposed to radical scavengers by providing hydrogen atom or electron to be a stable diamagnetic molecule. The scavenging effect rapidly increased from 0.5 mg/ml to 8.0 mg/ml (Fig.3). At 8.0 mg/ml the scavenging activity was highest (91.85 %) on DPPH radical. But at 0.125 mg/ml highest scav-

enging abilities of BHA and ascorbic acid were 97.83% and 95.37% respectively.

EC50, is the effective concentration at which the antioxidant activity was 50% and DPPH radicals were scavenged by 50%. EC50 was obtained by interpolation from linear regression analysis. Methanolic extract of *S. commune* showed significant scavenging effect of EC50 value at 1.4 mg/ml (50%).

Total phenol content

Total phenol content in *S. commune* was 2.0 mg/g.

The total protein content of *S. commune* was 15.55% which was much lower than the record of Kumar *et al.* (2013) but total carbohydrates and crude fiber content were much higher. Protein, ash and fat content were very much lower but carbohydrate content was much higher than the record of Amrita *et al.* (2016). Crude fat content was 9.0% in *S.commune* which was closely similar with the finding of Colak *et al.* (2009). Due to the presence of different nutrients in *S. commune,* it can be easily include as human dietary food.

Fig. 1 : Mature fruit body of *S. commune*, A. Upper view of mature fruit body. B. Lower view of mature fruit body

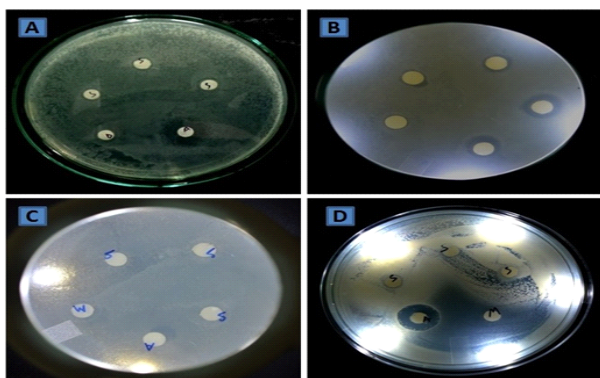

Fig. 2: Antibacterial property of *S. commune* against *Bacillus subtilis* (A), *Xanthomonas campestris* (B), *Staphylococcus aureus* (C) and *Escherichia coli* (D)

Temperature was found to be an important environmental factor that controls the growth. The optimum temperature for the growth *S. commune* was 25°C, which was at par with the record of Nasreen *et al.* (2015) and Adejoye *et al.* (2007). Wild edible mushroom of *S.commune* produced maximum mycelial biomass at pH 5.5 (Ogunjobi *et al.* 2007; Adejoye*et al.,* 2007 and Nasreen *et al.* 2015) but our findings showed that maximum mycelial biomass was produced at pH 6.0. This result revealed that test mushroom required slightly acidic pH for maximum mycelial biomass production. The best carbon source was starch instead of mannitol, which was reported by Ogunjobi *et al.* (2007). As our findings, Barakat and Sadik (2014) also recorded starch as the best carbon source for optimum biomass production in *S. commune*. But Nasreen *et al.* (2015) documented glucose and sucrose as favorable carbon sources. Mannitol and sorbitol are good carbon sources for mycelial growth of *S. commune* (Adejoye *et al.* 2007). Present results revealed that the best organic and inorganic nitrogen sources were yeast extract and ammonium sulphate respectively. According to Das *et al.* (2015), the yeast extract were the best nitrogen source of growth of *Lentinus squarrosulus,* which showed similarity with our finding. According to Nasreen *et al.*(2015), ammonium sulphate and ammonium nitrate were the best inorganic nitrogen sources for the maximum mycelial biomass yield of *S. commune*, which was similar with our findings.

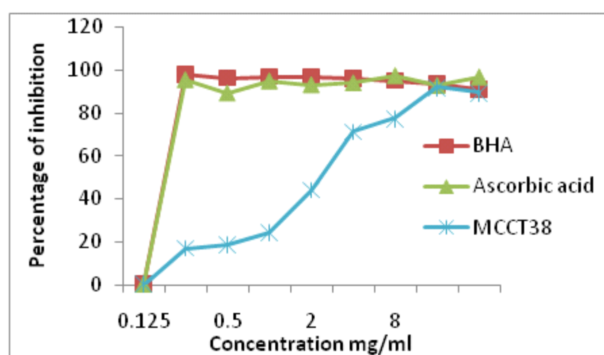

Fig. 3: Antioxidant activity of methanolic extract from dried mushroom *S. commune* (MCCT 38)

Giri *et al.* (2012) found substantial antimicrobial activity from several Basidiomycetes species. Antimicrobial activity was noticed from several species of *Lactarius* (Barros *et al.* 2007), *Fomitopsis*, *Cortinarius* and *Boletus* (Bala *et al.* 2011). The scavenging ability of mushroom extract on DPPH

depends on type of solvent used and showed ability in the order like methanol > ethyl acetate > hot water (Sudha *et al.* 2012). Devi *et al.* (2014) showed that ethanolic extraction of *S. commune* had an EC50 value of 0.883 mg/ml which was slightly lower than our value. It was reported that the EC50 value of edible mushrooms such as *Agaricus bisporous* and *Agaricus brasiliensis* in different solvent extraction were 1.67 and 4.57 mg/ml respectively (Gan *et al.,* 2013). Arbaayah and Kalsom (2013) determined that the IC50 (EC50) value at different flush of cultivated *S. commune* ranged from 2.75 mg/ml to 3.70 mg/ml. It has been reported that the antioxidant activity of plant material is strongly correlated with the phenolic content (Yap *et al.* 2014; Cheung *et al.* 2003; Mau *et al.* 2004). Babu and Rao*,* (2013) reported that total phenol content were in the range of 14.73–26.72 mg/g in different commercially cultivated Indian edible mushrooms, which is very much higher than our record.

The evaluation of different physical and chemical requirements for optimum biomass production by *S. commune* shall provide some basic information regarding the requirements of commercial cultivation and the nutraceutical properties could be exploited for providing better health status of the consumers.

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