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Research Article

ANTIOXIDANT AND PREBIOTIC POTENTIAL OF CULTURED MUSHROOM (*AGARICUS BISPORUS*) EXTRACT

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ABSTRACT

Gastrointestinal tract performs many functions in the body such as digestion, absorption, assimilation of the food; protection against pathogens and boosts immune system. Diseases of gastrointestinal tract have been majorly associated with improper dietary habit and lifestyle. Beneficial microorganisms (probiotics) in the gut play important nutritional and physiological roles. These microorganisms fed on prebiotics. Prebiotics are supplements or foods that contain food ingredients that selectively stimulate the growth of probiotics. Mushrooms have high protein content, vitamins, fibers, minerals, trace elements, and low calories and they lack cholesterol. Mushrooms also rich in polysaccharides, especially glucans, can stimulate the immune system.

In this present investigation prebiotic potential of cultured mushroom (*Agaricus bisporus*) was estimated by studying the growth of *Lactobacillus acophidophilus* and *Bifidobacterium longum* in the presence and absence mushroom extract and results showed dose dependent stimulatory activity on the growth and growth rate of *Lactobacillus acophidophilus* and *Bifidobacterium longum*. Phytochemical (qualitative and quantitative) analysis revealed that mushroom was rich of phenols and flavonoids. Mushroom extract also showed strong DPPH-free radical scavenging activity and hydroxyl radical inhibitory potential. From this observation it can be stated that mushroom extract posses strong prebiotic potential as well as anti-oxidant property. As polyphenols and their metabolites posses strong prebiotic potential as well as anti-oxidant capacity, it can be concluded that rich source of wide range of polyphenol can be the probable cause of prebiotic as well as anti-oxidant potential of mushroom.

Keywords: Prebiotics, Mushroom, *Lactobascillus sp, Bifidobacterium sp,* Anti-oxidant.

1. INTRODUCTION

For countless ailments mushrooms have been used for several thousands of years. Initially, mushrooms were known to be only a source of food but, later, their medicinal properties were discovered [1]. The use of mushrooms dates back to the ancient Egyptians and ancient Chinese cultures to promote general health and longevity. The early record of the Materia Medica shows evidence of using mushrooms for treating diseases. The number of mushrooms identified to date represents only 10% of total mushrooms assumed to exist [2]. Mushrooms rich in polysaccharides, especially glucans, can stimulate the immune system. Mushrooms have high protein content (up to 44.93%), vitamins, fibers, minerals, trace elements, and low calories and they lack cholesterol [3]. Mushrooms offer significant vital health benefits, including cholesterol-lowering properties, anti-hypertensive, anti-inflammatory, liver protection, as well as anti-diabetic, anti-viral, and anti-microbial properties [4].

During recent decades, there has been an increased awareness of food effects on health. Several epidemiological studies have demonstrated that functional foods reduce the risk of cancer, improve heart health, enhance the immune system and improve gastrointestinal (GI) health [4]. The concept of functional food includes prebiotics, defined as non-digestible food ingredients stimulating the growth of beneficial bacteria in the GI tract. The main criterion for potential prebiotic agents is that they must pass to the large intestine without being digested or absorbed in the upper GI tract, so that they become accessible for probiotic bacteria [5, 6]. Prebiotics have been found to reduce the incidence and duration of intestinal infections, down-regulate allergic response, improve digestion and improve the elimination of faeces. Prebiotics' beneficial effect is

associated with changes in the composition of GI microbiota. Positive prebiotic activity of some substances results in significant increases in the numbers of *bifidobacteria* and *lactobacilli* [7, 8]. These two genera exert wide range activity; *bifidobacteria* have been shown to stimulate the immune system, restore the normal flora after antibiotic therapy, inhibit pathogen growth and produce vitamin B, while *lactobacilli* help to digest lactose in lactose-intolerant individuals, as well as reduce constipation, diarrhoea or irritable bowel syndrome [9, 10].

2. MATERIAL AND METHODS

2.1. Collection of samples

The samples (cultured mushroom: *Agaricus bisporus*) were bought from the super market of Kolkata, West Bengal; India.

2.2. Preparation of Mushroom extract

Cultured mushroom (*Agaricus bisporus*) was cut into small pieces and sun dried for 2 days. Afterwards 5g of sundried mushroom were dipped in 10 ml of ethanol and incubated for 48 hours. Finally, filtrate was taken and stored for experiments. For prebiotic study, the mushroom extract was further diluted with distilled water (1:1).

2.3. Prebiotic potential

2.3.1. Culturing of Lactobacillus Acidophilus and Bifidobacterium Longum.

Lactobacillus acidophilus (NCDC 295) and *Bifidobacterium longum* (ATCC15707), both were cultured MRS broth. The solution was mixed thoroughly and kept in an incubator at 37°C for 24 hours. The cultures were maintained in a usable form by giving re cultures regularly to avoid death of the microorganisms.

2.3.2. Assessment of the growth of Lactobacillus Acidophilus and Bifidobacterium Longum. in presence and absence of mushroom extract

2.3.2.1. Colony count

Different concentrations of mushroom extract (0.1, 0.2, 0.3 & 0.4 ml) were added in petri plate except control. (In control plate only the equal volume of solvent of mushroom extract was added). The petri plates were prepared by pour plating (either with *Lactobacillus acidophilus* and *Bifidobacterium longum*) and were incubated at 37°C. Microbial growths were observed after 24 hours and number of colony was also counted.

2.3.2.2. Estimation of growth rate of Lactobacillus Acidophilus and Bifidobacterium Longum. in presence and absence of mushroom extract

MRS broth was poured into three clean 500 ml clear glass Nephelo culture flasks and labelled as follows: Control, Test 1 (0.3ml mushroom extract), Test 2 (0.4 ml mushroom extract). In 'control' plate, culture and 0.4 ml solvent of mushroom extract were added. Whereas in the 'test' flask culture and mushroom extracts were added. Flaks were cotton plugged and kept in the incubator at 37°C. Flasks were taken out at different time intervals to measure optical density. This was continued up to 9 hours [11].

2.4. Phytochemical analysis of Mushroom

2.4.1. Qualitative analysis of the phytochemical content of mushroom extract

Chemical tests for the screening and identification of bioactive chemical constituents in the mushroom were carried out with the extracts using the standard procedure as described. For each test, 1 ml of each solvent extract was used for analysis, in exception for the saponin test in which 3 ml solvent extract was used [12].

2.4.2. Determination of phenol content of mushroom extract:

The total phenol content of mushroom extract was determined using an UV/VIS spectrophotometer (Perkin Elmer) and Folin Ciocalteau's reagent. The Folin reagent was first prepared at a dilution of 10% using distilled water and 5 ml of the guava leaf extract was mixed. Thereafter, anhydrous sodium carbonate was prepared at a concentration of 75%, and 4ml of the mixture was added to the Folin mixture, hence producing a blue coloured solution. A blank was prepared and the resulting mixtures were shaken vigorously and incubated at 40°C for 30 minutes. The resulting optical densities were measured at 765 nm using a spectrophotometer. The absorbance against mg of gallic acid was measured and the graph obtained was used to determine the phenol content of guava leaf extract [12].

2.4.3. Determination of total flavonoids content

The total flavonoids contained in the extracts were estimated following the procedure of Ordon-ez et al [13]. Briefly, 0.5 mL of 2% $AlCl_3$ ethanolic solution was added to 0.5 mL of the extracts, left for 1 h at 25°C; after which the absorbance was measured at 420 nm.

The appearance of yellow colour suggested the presence of flavonoids. Extracts sample was evaluated at final concentration of 1 mg/mL. Total flavonoid content was expressed as quercetin equivalent (mg/g) using the equation obtained from the calibration curve.

2.5. Antioxidant property of mushroom:

2.5.1. DPPH radical scavenging assay

The antioxidant activity of the mushroom extract was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, as described by Turkoglu et al [14]. In brief, 1 mL of a 0.2 mM DPPH methanol solution was added to 1mL of various concentrations (0.2-1.0 mg/mL) of the extracts and incubated at 25°C for 30 min. The absorbance of the resulting mixture was measured against blank at 516 nm.

2.5.2. Hydroxyl radical inhibitory potential

Forty mL of the freshly prepared extracts (0.2-1.0 mg/mL) was added to a reaction mixture containing 20 mL 20 mM deoxyribose, 80 mL of 0.1 M phosphate buffer, 10 mL of 500 mM $FeSO_4$, and the volume was made up to 200 mL with distilled water. The reaction mixture was initiated at 37°C for 30 min, and stopped by adding 50 mL of 2.8% TCA (trichloroacetic acid). This was followed by the addition of 50mL of 0.6% thiobarbituric acid solution. The mixture was then incubated in boiling water for 20 min and absorbance was read at 532 nm [15].

2.6. Statistical analysis

The results of experiments were expressed as mean ± standard deviation of triplicate analyses.

3. RESULTS AND DISCUSSION

3.1. Growth of *Lactobacillus acidophilus* and *Bifidobacterium longum* in presence and absence of mushroom extract

Lactobacillus and *Bifidobacteria* were grown in presence of different concentrations of mushroom (0.1, 0.2, 0.3, 0.4 ml) and colony count was done after 24 hours (table 1). It was found that mushroom extract stimulates the growth of *Lactobacillus* and *Bifidobacteria* as the colony count is much higher in mushroom extract treated plate compared to control. It was also observed that stimulatory effect of mushroom extract on *Lactobacillus* was much higher compared to *Bifidobacteria*.

Table 1: Colony count of *Lactobacillus Acidophilus.* and *Bifidobacterium Longum.* in presence and absence of mushroom extract

Mushroom Sample	Number of colonies (*Lactobacillus Acidophilus*)
Mushroom Extract (0.1 ml)	456 CFU/ml
Mushroom Extract (0.2 ml)	750 CFU/ml
Mushroom Extract (0.3 ml)	980 CFU/ml
Mushroom Extract (0.4 ml)	1020 CFU/ml
Control	325 CFU/ml
Mushroom Sample	**Number of colonies** (*Bifidobacterium Longum*)
Mushroom Extract (0.1 ml)	325 CFU/ml
Mushroom Extract (0.2 ml)	581 CFU/ml
Mushroom Extract (0.3 ml)	625 CFU/ml
Mushroom Extract (0.4 ml)	750 CFU/ml
Control	205 CFU/ml

3.2. Growth rate of *Lactobacillus acophidophilus* and *Bifidobacterium longum* in presence and absence of mushroom extract

Lactobacillus and *Bifidobacteria* were grown in presence and absence of mushroom abstract and optical density was measured every hour after inoculation upto 9 hours (fig. 1). It was observed that growth rate of *Lactobacillus* and *Bifidobacteria* were much higher in presence of mushroom extract compared to control (fig. 1).

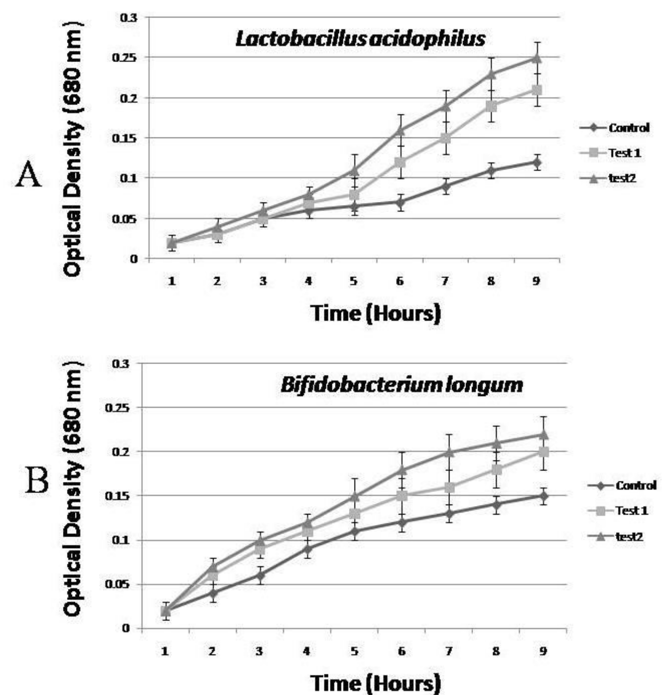

Fig. 1: Growth rate of *Lactobacillus acophidophilus* and *Bifidobacterium longum* in presence and absence of mushroom extract. [Test 1: (0.3ml mushroom extract), Test 2: (0.4 ml mushroom extract)]

Results of the above experiments showed that mushroom extract has stimulatory effect on the growth and growth rate of *Lactobacillus Acophidophilus.* and *Bifidobacterium Longum.* Colony count also showed that stimulatory effect of mushroom extract on *Lactobacillus* was higher compared to *Bifidobacteria*.

3.3. Qualitative analysis of the phytochemical content of mushroom extract

Result of qualitative phytochemical analysis of mushroom extract showed that mushroom was rich in phenol and flavanoids, although some amount of anthraquinones and alkaloids were pesent (table 2)

Table 2: Phytochemical analysis of mushroom extract (qualitative)

Compound tested	Result
Anthraquinones	+
Flavonoids	++
Phenols	+++
Saponin	-
Tannins	-
Alkaloids	+

Absent, + present, ++ moderate, +++ high

3.4. Determination of total phenol and flavonoid content of mushroom extract

As mushroom extract was rich in phenol and flavanoid, total phenol and flavonoids content of mushroom extract were quantified photometrically and found that very high concentration of phenol and flavanoids were present in mushroom extract (table 3).

Table 3: Total phenol and flavonoid content of mushroom extract

Parameters	(µg/100g)
Phenols	172.9±9.8
Flavonoids	91.8±6.7

Phytochemical analysis also showed that mushroom extract was rich in phenol and flavanoids. It is known that dietary polyphenols contribute to the maintenance of gastrointestinal health by interacting with epithelial cells and largely by modulating the gut microbiota composition. Polyphenols may act as promoting factors of growth, proliferation, or survival for probiotics mainly *Lactobacillus* strains and thus exerting prebiotic actions and inhibiting the proliferation of pathogenic bacteria such as *Salmonella* and *Helicobacter pylori* [16]. In addition, polyphenols may be converted by the colonic microbiota to bioactive compounds that can affect the intestinal ecology and influence host health. There is evidence from *in vitro* animal and human studies that certain doses of selected polyphenols may modify the gut microbial composition, and while certain bacterial groups can be inhibited, others can thrive in the available niche of the ecosystem.

Animal experiment found that when rats were given a tannin-rich diet, the Bacteroides group increased significantly while the *Clostridium leptum cluster* decreased significantly [17]. Dolara et al. reported that, when rats were treated with red-wine polyphenols, they had significantly lower levels of *Clostridium spp.* and higher levels of *Bacteroides, Bifidobacterium* and *Lactobacillus spp*. [18]. Similarly, the resveratrol commonly found in grape promoted faecal cell counts of *Bifidobacterium spp.* and *Lactobacillusin* in a rat model [19].

In the study by Goto et al., the polyphenols present in tea has a positive effect on the growth of *Bifidobacterium spp.* Decline in the number of putrefactive bacteria like *Enterobacteriaceae spp.* and *Clostridium spp.* was noticeable as well. The consumption of tea evidently improved conditions in the intestines by lowering the levels of sulfides, ammonia, and pH [20]. Tzounis et al. evaluated the influence of flavonols derived from cocoa with the potential of being used as prebiotics. Patients were divided into two groups where the first one obtained 494 mg of cocoa flavonols per day and the other group was given 23 mg of the same flavonols daily for the period of four weeks. Researchers determined a statistically significant growth of *Bifidobacterium* and *Lactobacillus*. Both types of bacteria inhibit development of pathogenic microorganisms [21].

3.5. Antioxidant property of mushroom exract

To evaluate the anti-oxidant property DPPH free radical scavaenging property and hydroxyl radical inhibitory potential of mushroom were estimated and found that mushroom posses strong DPPH radical scavenging and strong hydroxyl radical inhibitory property (table 4).

Table 4: Antioxidant property of mushroom extract

Parameters	Percent (%)
DPPH radical scavenging activity	45.23±3.7
Hydroxyl radical inhibitory potential	90.12±9.6

Result also showed that mushroom extract posses strong anti-oxidant property, which is evident from its strong DPPH free radical scavenging activity as well as hydroxyl radical inhibitory potential. The antioxidant

efficiency of medicinal plants has generally been ascribed to their high phenolic contents [22, 23]. In the present study, the occurrence of phenolic compounds (phenols, flavonoids) in mushroom extract is suggestive of the mushroom as a potent antioxidant. For instance, phenols are well known antioxidants and cellular event modifiers [24], while flavonoids are powerful free radical scavengers with strong anticancer activity [25, 26]. Reactive oxygen species cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, and neuro-degenerative diseases. Phenolics are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes and inhibit oxidases and helps in the management of degenerative diseases, such as diabetes and hypertension [27]. H_2O_2 is an important reactive oxygen species based on its ability to cause oxidative degradation of cell membrane lipids to give rise to the occurrence of mutagenesis and cytotoxicity. It is rapidly broken down into water and oxygen, thereby producing hydroxyl radical that can initiate lipid peroxidation and cause DNA damage in the body [27]. And mushroom extracts also showed strong hydroxyl radical inhibitory potential.

4. CONCLUSION

It can be concluded from the above observation that cultured mushroom possess strong prebiotic potential as well as anti-oxidant property. Prebiotic potential of mushroom is evident from its stimulatory effect of the growth of *Lactobacillus acophidophilus* and *Bifidobacterium longum* Phytochmical analysis also showed that mushroom is rich in phenol and flavaoids. As phenolic compounds may act as promoting factors of growth, proliferation, or survival for probiotics, so we can conclude that rich polyphenolic composition may be the probable cause of prebiotic potential of mushroom. The antioxidant efficiency has generally been ascribed to their high phenolic contents and mushroom showed strong anti-oxidant property as evident from DPPH free radical scavenging activity as well as hydroxyl radical inhibitory potential. Anti-oxidant potential can give protection against oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, and neurodegenerative diseases. Overall, mushroom can be considered as new superfood and can be considered as vital component of human diet for improving health and promoting quality of life. As a result of these properties, mushrooms extracts as well as powders can used for the prevention and treatment of various life threatening diseases and also can be used as nutraceuticals or dietary supplements.

Conflict of interest

There is no conflict of interest regarding publication of this paper.

5. ACKNOWLEDGEMENT

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