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Phytochemical analysis and Protein quantitation and profiling of some commercially cultivated Mushroom species

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Abstract

Mushroom can be defined as a macrofungus with a distinctive fruiting body. Mushrooms are recognized as natural and healthy foods and credited to be the third largest macro-fungus cultivated for food and industrial purposes worldwide. Besides containing high protein, vitamins, fibers and low calories, it also has many medicinal properties. In this study, ten samples of mushrooms were screened for phytochemicals such as Cardiac glycosides, Anthraquinones, Terpenoids, Proteins, Flavonoids, Saponins, Tannins, Lignins and Phenol. All the samples showed positive result for Terpenoids and Proteins and showed negative result for Anthraquinones, Flavonoids, Tannins, Lignins and Phenol. Most of the samples found positive for Cardiac glycosides and Saponins. Protein estimation was done by Bradford's assay and concentration found was between 0.7572 mg/ml (White Beech) to 2.5972 mg/ml (King Oyster). Crude protein extract was separated on 12% polyacrylamide gel and clear distinct band was observed with silver staining. Total bands were varied sample to sample ranging from 9 to 20 bands and different sizes from 10 kDa to 130 kDa. The described approach holds great promise for further analyses and gives support to discrimination as well as for conservation of protein resources.

INTRODUCTION

Mushrooms are considered to as nutritious food and used for the prevention and treatment of diseases [1]. Mushrooms are defined as macrofungi which belongs to the class Ascomycetes [2] with a distinctive fruiting body that may be above or below ground and can be seen with our naked eyes [3]. Moreover, mushrooms have been employed as a "medicine" [4] and it's strongly connected with our life and is valuable for scientific study and research [5]. The medicinal use of mushrooms has a very long tradition in the Asian countries, whereas their use in the Western hemi-sphere has been slightly increasing only since the last decades.

Mushroom cultivation is practiced in more than 100 countries and its production is increasing at the rate of 7 percent per annum. Mushroom production has already crossed 5 million metric tons annually in the world and is expected to reach around 7 million metric ton in next ten years. India had been known world over for its 1.5% contribution to the worldwide mushrooms production. Total mushroom production in India was 40,600.00 tons in 2009-10. The market value of medicinal mushrooms and their derivative dietary supplements world-wide was US $1.2 billion in 1991 [6] and was estimated to be US $6 billion in 1999 [7]. 'Mushroom' is not a taxonomic category. The term 'mushroom' should be used here according to the definition of Chang and Miles as 'a macrofungus with a distinctive fruiting body, which can be hypogenous or epigeous, large enough to be seen with the naked eye and to be picked by hand' [8].

Mushrooms have been consumed by humans since ancient times not only as a part of the normal diet but also for its desirable taste. Reference to mushrooms is found in Vedas [9, 10]. Among natural products, mushrooms have been recognized as potent candidates in clinical studies because they are readily obtained in relatively large quantities and are inexpensive [11]. The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than wheat [12]. Due to their rich content of proteins, vitamins and minerals mushrooms are considered to be as "Poor man's Protein" [13]. Mushrooms can be used for the food to solve the malnutrition problem [14]. Other than proteins and vitamins mushroom species such as Pleurotus ostreatus [15], Pleurotus tuberregium [16], Lentinus edodes [17], Irpex lacteus [18] and Volariella olacea [19] are also found to produce an important enzyme RNases.

Mushroom lectins have captured the attention of some investigators on account of the antiproliferative, antitumor, mitogenic, immunomodulatory, hypotensive and vasorelaxing, and anti-HIV-1 reverse transcriptase activities that they exhibit [20-22]. It is also reported that Mushrooms are a source of physiologically beneficial and non-toxic medicines, low in calories and high in minerals, essential amino acids, high proteins, vitamins and fibers [23-26] as well as nutritionally functional food. The high proteins, sterols, macro-elements and low calorie content make mushroom ideal for prevention of cardiovascular diseases [27].

A food or part of a food that provides medical or health benefits like the prevention and treatment of disease can be considered as a nutraceuticals [28]. Mushrooms are proven to be richest sources of nutraceuticals [29-31] and their bioactive properties [32]. In this study the evaluation of these nutraceutical composition such as Cardiac glycosides, Anthraquinones, Terpenoids, Proteins, Saponins, Tannins, Lignins and Phenol in ten mushroom species was assayed, and protein profiling has been done by SDS-PAGE.

MATERIALS AND METHODS

Sample Collection

Total ten samples of mushroom viz. Shitake, Brown Shimeji, Pink Oyster, Button Mushroom, White Beech, White Shimeji, Enok, Oyster, Protobello and King Oyster were collected from local markets of Bangalore.

Preliminary Phytochemical Analysis

The mushroom samples were air dried at room temperature and powdered and used for screening of phytochemical analysis. The ten powdered samples were extracted for 48 hours with methanol in the ratio of 1: 10. According to Harbone [33] phytochemical screening of mushroom extracts was carried out for the presence of Cardiac glycosides, Anthraquinones, Terpenoids, Proteins, Flavonoids, Saponins, Tannins, Lignins and Phenol.

Extraction of Protein

Collected mushroom samples were washed and weighed. Total protein extraction was carried out as follows: 1 gram of each sample was homogenized using a clean mortar and pestle with 4-5 ml of freshly prepared 50 mM Phosphate buffer (pH-7.2). Homogenized samples were centrifuged at 8000 RPM for 10 min to remove debris. Without disturbing pellet upper clear supernatant was transferred to a fresh tube and stored at 4ºC. Relative or Absolute amounts of protein in the extracts was determined using Bradford's protein estimation method [34].

SDS-PAGE

Protein profiling was done for ten mushroom samples by Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE). 12% polyacrylamide gel was prepared according to standard Laemmli 1970 method [35] with slight modification. Protein samples were prepared by mixing 30µl of protein sample with 30µl of sample loading buffer and heated at 95°C for 5minutes to denature the polypeptide chains. About 50 µl of the denatured protein samples were loaded into the wells of the gel. The proteins were allowed to run at 60 volts in 1x SDS Running buffer. The separated proteins were stained by standard silver nitrate staining methods and bands were observed.

RESULTS

Selected samples of mushrooms were screened for the phytochemical properties of Cardiac glycosides, Anthraquinones, Terpenoids, Proteins, Flavonoids, Saponins, Tannins, Lignins and Phenol. Among ten samples screened, all mushroom samples were tested positive for terpenoids and proteins. Cardiac glycosides were found to be present in all except Button mushroom, White beach, Enok and Portobello. Saponin was present in Shitake, White beach, Enok, Oyster Portobello and King oyster mushroom. The test for Anthraquinones, Flavonoids, Tannins, Lignin and Phenol were negative for all mushroom samples. The result of preliminary phytochemical analysis is shown in table 1. By Bradford's method, the total protein content estimation of all mushroom samples has showed the maximum of 2.5972 mg/ml for the King oyster and the minimum of 0.7572 mg/ml for White beech variety, as shown in table 2.

As shown in fig. 1, Protein profiling studies by 12% SDS-PAGE by silver staining has showed approximately 9 to 35 bands. A thick unique band was observed at 39kDa for the Enok, at 37kDa for Pink oyster and at 70kDa for Shitake mushroom. King oyster was found to have more number of bands between the molecular range 39kDa and 90kDa. Though the protein concentration is nearly same for Brown Shimeji and White Shimeji, significant difference was observed in their banding pattern.

Table 1. Preliminary phytochemical analysis of screened mushroom samples.

Test/Sample	Shitake	Brown Shimeji	Pink Oyster	Button Mushroom	White Beech	White Shimeji	Enok	Oyster	Portobello	King Oyster
Cardiac glycosides	+	+	+	-	-	+	-	+	-	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-	-	-
Saponins	+	-		-	+	-	+	+	+	+
Tannins	-	-	-	-	-	-	-	-	-	-
Lignins	-	-	-	-	-	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	-	-

Table 2. Quantitative analysis of protein by Bradford's assay.

S.No	Mushroom sample	Protein Concentration (mg/ml)
1	Shitake	1.3932
2	Brown Shimeji	0.9572
3	Pink Oyster	1.7672
4	Button Mushroom	1.8372
5	White Beech	0.7572
6	White Shimeji	1.0212
7	Enok	1.3612
8	Oyster	1.7832
9	Protobello	2.3932
10	King Oyster	2.5972

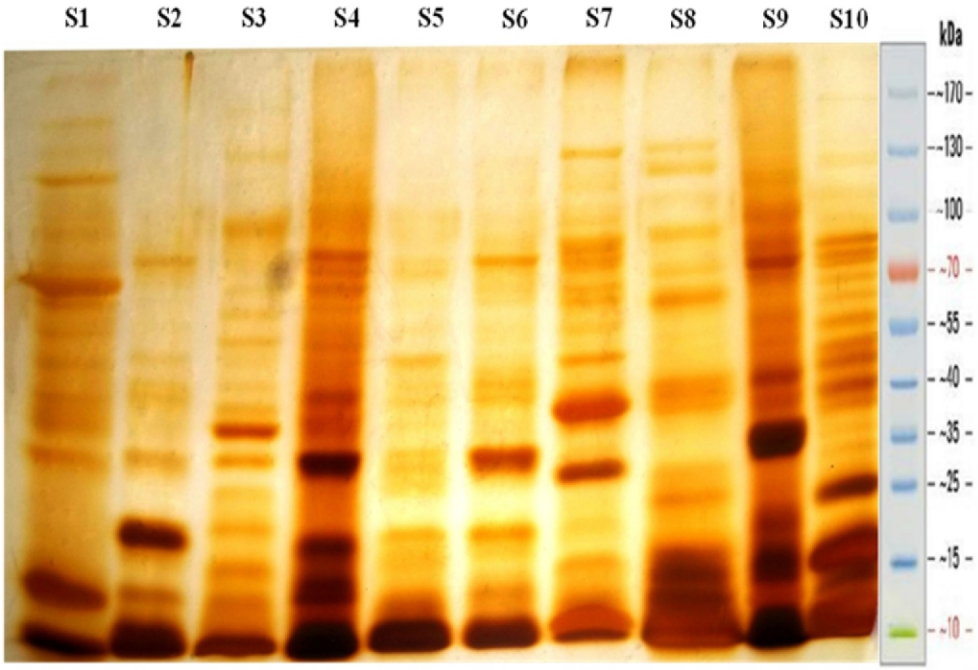

Fig 1. Protein profiling of Mushroom samples by SDS-PAGE

DISCUSSIONS

Recently there has been a renewed interest in improving health and fitness through the use of more natural products [36]. Phytochemicals are compounds that are used as food and medicine to protect against illness and to maintain human health [37]. In this study, Terpenoids and Proteins are found to be the major compounds for methanolic extracts of all mushroom varieties. High protein content in mushroom made it is regarded as an ideal protein source for vegetarian as well as for old age people. It was found that the total amount of protein varies from species to species in the same genus [38]. Supporting the previous studies [38] the total protein content has showed 0.7572 mg/ml to 2.5972 mg/ml of protein, these results prove that mushroom is an abundant source of protein which is economically available. SDS-PAGE analysis of the ten samples has showed numerous banding patterns in the 12% polyacrylamide gel. Proteins obtained were spread uniformly between the molecular weight 10 kDa to 160 kDa. Confirming with the protein concentration the species King oyster, Protobello, Oyster and Pink oyster has showed the more number of bands. White beech and Brown

shimeji has showed minimum number of plants.

CONCLUSION

The wild edible macrofungi has shown to be as a richest source of proteins with the maximum concentration of 2.597mg/ml. Thus our results suggest that the Proteins and phytochemicals rich mushrooms can serve as a food and medicine. Further work is needed to study its useful antagonistic and antioxidant properties.

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