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Proximate Analysis, Phtochemical Screening and Antioxidant Activity Of Different Strains of *Auricularia auricula-judae* (Ear Mushroom)

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ABSTRACT

In this study, proximate analysis, phytochemical screening and antioxidant activity of two strains of ear mushroom Auricularia auricula (arbitrarily named strain 3 and 5) and their mix, cultivated in Bangladesh National Mushroom Development Institute, have been determined. Protein content per 100 gm of strain 5, 7 and mix had been found to be 298.69mg, 278.85mg and 286.19mg, respectively. Lipid content estimated were 2.43gm, 1.96 gm and 2.4gm, respectively, while that of ash were 4.42 gm, 6.11gm and 3.93gm, respectively. A. auricula strain 7 contained highest amounts of total phenol, total flavonoid, ascorbic acid and reducing sugar than the others. Among the three strains evaluated in the present study, A. auricula 7 contained highest nutritional and medicinal components. Thus, A. auricula 7 might be an ideal food supplement to the consumers.

Keywords: Anti-oxidant; Auricularia auricula; Phytochemical analysis; Proximate composition.

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Introduction

Auricularia auricular-judae is the mushroom of auricularia genus. These mushrooms are commonly called ear mushroom due to their ear like appearance.Their fruting body is brown in coloration and grows upon wood which is especially damp and elder. *A. auricula* (common names: mu-er, wood ear), are heterobasidiomycetes, in the order Auriculariales and the family Auriculariaceae. *A. auricula* was the first artificially cultivated mushroom in China. *Auricularia* mushrooms are among the top four most important cultivated mushrooms in the world, grown mainly in China and Southeast Asia, with a world annual production of 420,000 tons (Luo,et al, 2004).Their unique jelly texture and horizontally septated basidium make them significantly different in taste and morphological characters from other cultivated mushrooms, such as *Agaricus bisporus, Lentinula edodes, Pleurotus* spp., and others. Besides their taste and nutrition, they also have medical functions, such as anti-tumour, immuno-stimulating, hypolipidaemic and hypocholesterolaemic effects (Ukai et al. 1983; Cheung 1996; Song et al. 1998; Yang et al. 2002). They can grow well on wide ranges of agricultural and industrial organic wastes. Recently, a nature-imitated cultivation method has been developed in China by cultivating *Auricularia* mushrooms in corn fields, and thereafter, using the spent compost as organic fertilizer and soil conditioner (Yan et al. 1995).

Though the usage of mushrooms is being increased day by day for their significant role in human health, nutrition and disease, in Bangladesh, mushrooms are considered as important food items. People in Bangladesh are still not very aware of nutritional and medicinal importance of mushrooms. Climatic condition of Bangladesh favors the easy cultivation of ear mushroom, *A. auricula.* Yet, there is scarcity of interest of common mass in cultivating and including this mushroom in daily diet. Thus, the present study has been aimed at determining the nutritional and medicinal values of *A. auricula* so that the consumer would be aware of its nutritional and medicinal values and consider this mushroom among the functional food items.

Materials and methods

Mushroom collection and preparation

Fruiting bodies of *A. auricula* were collected from Bangladesh national mushroom development institute and cut into small pieces. Small pieces were dried under the sun followed by hot air oven at the temperature 55°C until proper drying. After drying, the dried chips were ground into coarse powders using blender having high capacity grinding power. Then the powder were stored in air tight container with necessary markings for identification and kept in cool, dark and dry place for further investigation. Hot water extract (HWE) of *A. auricula* was prepared following the method of Rahman et al. (2016) [4].

Proximate analysis

Following the procedure established by the Association of Official Analytical Chemists (AOAC), the analyses were performed [5]. Analyses included the determination of crude protein, crude fat, ash, crude fiber, moisture and carbohydrate. The percentage of all the fractions (crude protein, crude fat and ash) were added together and subtracted from 100 to obtain the total carbohydrate percentage.

Antioxidant studies

Qualitative screening for antioxidant activity

Determination of phenols (Ferric chloride test)

Following the method of Soloway and Wilen (1952) [6], ferric chloride test was performed to assess the phenolics in the HWE of *A. auricula*.

Determination of flavonoids (Alkaline reagent test)

Following the method of Rahman et al. (2017) [7], Alkaline reagent test was performed to assess the flavonoids in the HWE of *A. auricula*.

Determination of ascorbic acid

Following the method of Schmall and Pifer (1953) [8], HWE of *A. auricula* was assessed for the presence of ascorbic acid.

Quantitative estimation of phytochemical constituents

Total phenolics content (TPC) assay

Following the modified method of Singleton et al. (1999) [9], content of total phenolics in the HWE of *A. auricula* was performed.

Total flavonoid content (TFC) assay

Following the modified method of Chang et al. (2002) [10], content of total flavonoids in the HWE of *A. auricula* was performed.

Ascorbic acid content assay

Following the modified method of Omaye et al. (1979) [11], content of ascorbic acid in the HWE of *A. auricula* was performed.

Total protein content assay

Following the modified method of Lowry et al. (1951) [12], content of total protein in the HWE of *A. auricula* was performed.

Reducing sugar content assay

Following the modified method of Nelson-Somogyi (1944) [13], content of reducing sugar in the HWE of *A. auricula* was performed.

Antioxidant assay

Ferric reducing antioxidant power (FRAP) assay

Following the modified method of Benzie and Strain (1996) [14], ferric reducing antioxidant power (FRAP) assay of the HWE of *A. auricula* was performed.

DPPH (1,1-diphenyl-2-picryl-hydrazyl) Free radical scavenging activity assay

The ability of the HWE of *A. auricula* to scavenge 2,2diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined according to the method Brand-williams (1995) [15] with little modification.

Results and Discussion

Proximate analysis of *A. auricula*

TABLE 1: Proximate analysis of *A. auricula* strains (%dry weight)

Proximate composition	*A. auricula* 3	*A. auricula* 5	*A. auricula* mix
Crude lipid	1.6±0.124	1.9±0.188	1.2±0.294
Crude protein	18.75±0.12	29.90±0.14	27.90±0.18
Fiber	22.8±0.106	19.14±0.260	21.8±0.241
Carbohydrate	51.65±0.13	37.18±0.15	38.91±0.17
Ash	3.77±0.0.121	3.69±0.249	3.96±0.187
Moisture	9.04±0.43	6.34±0.38	6.81±0.33

Results are expressed as mean±SEM.

The lipid content of 100 gm of dried *A. auricula 3, A. auricula 5, A. auricula mix* were found about 1.6gm, 1.9 gm and 1.2 gm respectively. The moisture content of *A. auricula* 3, *A. auricula* 5, *A. auricula* mix were found about 9.04%,6.34% and 6.81%respectively. The ash content of 100 gm of dried *A. auricula* 3, *A. auricula* 5, A. auricula mix were found about 3.77gm,3.69 gm and 3.96gm respectively. The fibre content of 100 gm of dried *A. auricula* 3, *A. auricula* 5, *A. auricula* mix were found about 22.8gm, 19.14 gm and 21.8gm respectively.

Phytochemical analysis of *A. auricula*

The maximum TPC was obtained in ethanolic *A. auricula* 5 extract (24.89 mg GAE/100g extract) at a concentration of 0.5 mg/mL. At the same concentration, the minimum TPC was observed in ethanolic *A. auricula* mix extract (20.45 mg GAE/100g extract). This report agrees to the one given by (Al-Juhaimi, 2014) that *A. auricula 5*

exhibit more phenol content than *A. auricula* 3 *and A. auricula mix* extract. The health benefits of polyphenolics are primarily derived from their antioxidant potentials because the radicals produced after hydrogen or electron donation are resonance stabilized and thus relatively stable (Denre, 2014). The use of phenolics is also reported for effective secretion of dopkine, lowering and preventing obesity and prevention of oxidative stress (Dalar et al., 2014). These polyphenolic compounds from natural sources are recommendable as natural food additives and they are considered more suitable for application in food products than butylated hydroxytoluene and butylated hydroxyanisole which are artificial compounds with antioxidant properties (Ghafoor et al., 2011). The maximum TPC was obtained in ethanolic Au 5 extract (24.89mg GAE/100g extract) at a concentration of 500 µg/mL. At the same concentration, the minimum TPC was observed in ethanolic Au-mix extract (20.45 mg GAE/100g extract).This report agrees to those of Al-Juhaimi (2014) who reported that *A. auricula* 5 exhibit more phenol content than *A. auricula* 3 *A. auricula* mix extract.

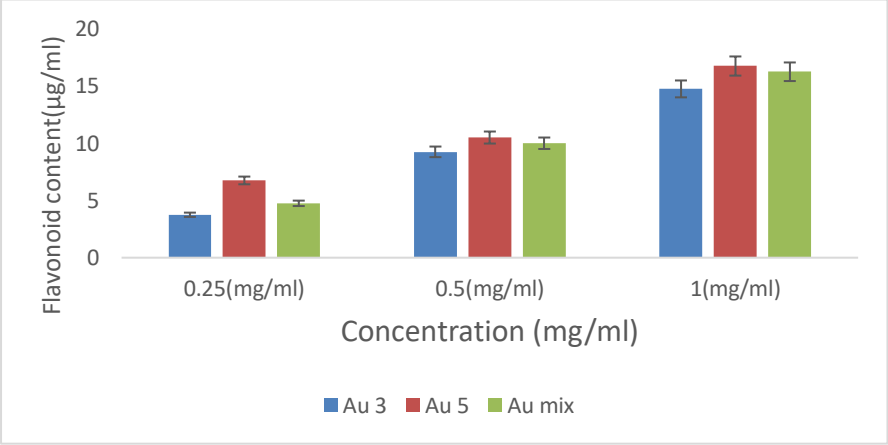

Figure 1: Total polyphenol content of *A. auricula* 3, *A. auricula* 5, A. auricula mix.

The content is expressed as milligram gallic acid equivalent per 100 gram in the *A. auricula* 3, *A. auricula* 5, *A. auricula* mix extracts which were prepared using polar solvent (ethanol) the extract. Total polyphenol content is expressed in mg GAE/100g extract. *A. auricula* 5 has more polyphenol content than other.

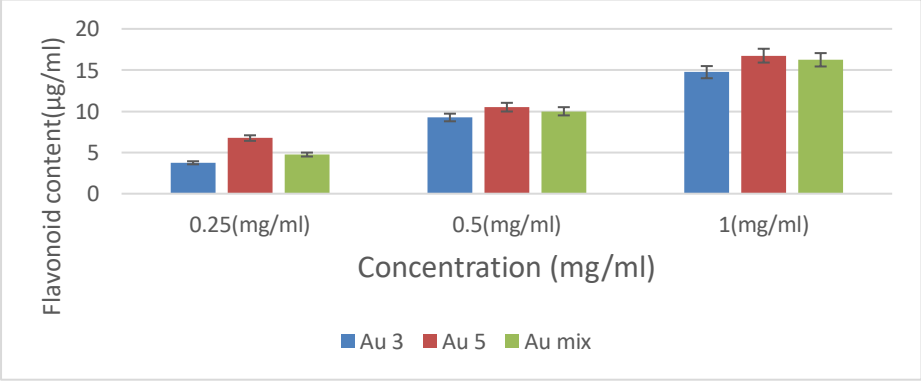

Figure 2: Total Flavonoid content of the *A. auricula 3, A. auricula 5, A. auricula mix* extracts.

The content is expressed as milligram catechin equivalent per 100 gram in the *A. auricula 3, A. auricula 5, A. auricula mix* extracts which were prepared using polar solvent (ethanol). Total flavonoid content is expressed in mg CE/100g extract. *A. auricula 5* showed presence of more flavonoid content than other.

Figure 2 revealed that the maximum TFC was observed in *A. auricula* 5 (10.5 mg CE/100g extract) at the concentration of 0.5 mg/mL where at the same concentration minimum TFC was observed in *A. auricular* 3 (9.25 mg CE/100g extract) which is supported by Levaj and co-workers (Levaj et al., 2009). Flavonoids are the plant pigments responsible for plant colors and exert their health-promoting activities through their high pharmacological potentials as radical scavengers (Cook and Samman, 1996). Flavonoids are the antioxidants that can prevent or delay the oxidation of substrates even when it is present in low concentrations, so as to prevent oxidation by the prooxidants (ROS and RNS). These non-enzymatic antioxidants (phenolics and flavonoids) react with the prooxidants leading to inactivation. In the redox reaction, the antioxidants act as reductants and serve as the first-line defense to suppress the formation of free radicals (Tilak et al., 2004). The flavonoids have strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens. Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. They have anti-allergic, antiinflammatory, anti-microbial and anti-cancer activity (Okwu et al., 2005).

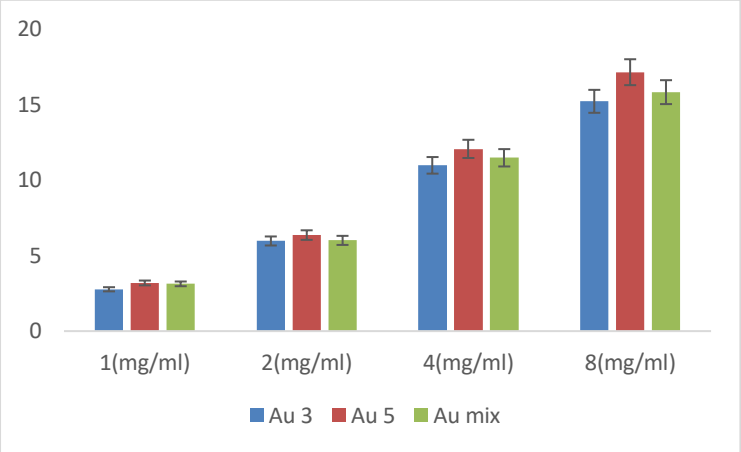

Figure 3: Ascorbic acid (vitamin c) content of *A. auricula 3, A. auricula 5, A. auricula mix* extracts.

The content is expressed as milligram ascorbic acid equivalent per 100 gram of *A. auricula 3, A. auricula 5, A. auricula mix* extracts which were prepared using polar solvents (Ethanol). Vitamin C content is expressed in mg AAE/100g extract. *A. auricula 5* showed greater presence of Vitamin C content than other extract.

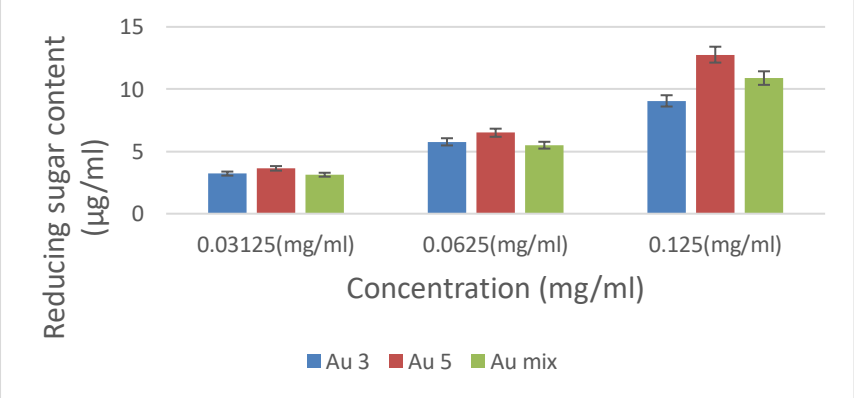

Figure 4: Reducing sugar content of *A. auricula 3, A. auricula 5, A. auricula mix* extracts. The content is expressed as miligram glucose equivalent per 100 gram of extracts which were prepared using polar solvent (Ethanol). Reducing sugar content is expressed in g GE/100g extract. *A. auricula 5* showed greater reducing sugar content than *A. auricula 3 and A. auricula* mix extract.

Reducing sugar is an important component in *A. auricula*. Ethanolic extract of *A. auricula 5* showed more reducing sugar (359.21mg GE/100g extract) than *A. auricula 3 and mix* (280.73gm GE/100g and 260.28mg GAE/100g extract) at the concentration of 0.125mg/ml. *A. auricula 5* contain more reducing sugar content than *A. auricula3,* and *A. auricula* mix. Carbohydrate-containing compounds are promising way to synthesize drugs that do not only save pharmacological properties of an initial agent but also acquire a number of advantageous features namely increased bioavailability, water solubility, and protection from quick metabolism in the body (Tolstikova et al., 2009).

The present study indicates that mushrooms are nutritious food item with a high protein and fibre content and less fat or lipid. Mushroom also contain a large amount of phytochemical and antioxidant. Nutritional content of *A. auricula* 3 and *A. auricula* 5 cultivated in Bangladesh vary from one another. The nutritional analysis of *A. auricula* 3 and *A. auricula* 5 separately has not yet published but the mix analysis has done. The protein content of *A. auricula* 5 is higher than the *A. auricula* 3 on average in this study is near about the data of Breene, W. M. (1990). Highest concentration of proteins would be the most beneficial to eat and the least damaging to tooth enamel. Therefore, higher the concentrations of proteins are digested to provide higher the quantities of amino acids which are being used by the body. However fibre, ash content were found higher in this study and carbohydrate and lipid content were found lower in comparison with the study of Chang, S. and J. Buswell (1996).This variation may be due to the different groth substrate, the method of cultivation, stage og harvesting and time interval between harvest and measurement methods, which interfers with the nutritional value of composition Kadnikova, I. A., R. Costa, et al. (2015). Mau et al. have found that among four commonly used medicinal mushrooms, *A. auricula* showed excellent antioxidant activity in a lipid system. Our experimental results showed that GLP is a very potent antioxidant with high antioxidant activity comparable to that of the synthetic antioxidant butylated hydroxytoluene (BHT).

The vitamin C is in *A. auricula* 5 extract is higher than the *A. auricula* 3 extract and *A. auricula* mix .On average this result is supported by Manning (1985). An important role for vitamin C as prevention or treatment for various diseases is disputed, with reviews reporting conflicting results. A review reported no effect of vitamin C supplementation on overall mortality (Buettner, G. R. and F. Q. Schafer 2004). Ascorbic acid is a strong antioxidant that directly interacts with a broad spectrum of harmful reactive oxygen species, terminates the chain reaction initiated by free radicals via electron transfer, and is involved in the regeneration of other antioxidants, to their functional state (Wu, x., diao et al 1999). Ascorbic acid deficiency can lead to anemia, scurvy, infections, bleeding gums muscle degeneration, poor wound healing, atherosclerosis plaques, capillary hemorrhaging and neurotic disturbances. Toxicity does not occur

Phenolic acids is the main phenolic compounds in mushrooms . According to Singleton et al, gallic acid, tannic acid, protocatechuic acid, and gentisic acids were some of the major phenolics detected in water extracts of several indigenous edible mushrooms from south asia. Besides, several authors have reported the correlation between the polarity of extraction solvent and phenolic content of resulting extracts. For instance, comparatively the phenolic content of extract in this study was determined in *A. auricula* 5 extract, which was higher than that of methanolic extracts of *A. auricula* 3 and *A. auricula* mix. The health benefits of polyphenolics are primarily derived from their antioxidant potentials because the radicals produced after hydrogen or electron donation are resonance stabilized and thus relatively stable .The use of phenolics is also reported, for effective secretion of dopkine, lowering and preventing obesity and prevention of oxidative

stress. These polyphenolic compounds from natural sources are recommendable as natural food additives and they are considered more suitable for application in food products than butylated hydroxytoluene and butylated hydroxyanisole which are artificial compounds with antioxidant properties (Crisan and Sands, 1978).

The phenolic content of extract in this study was determined in *A. auricula* 5extract, which was higher than that of methanolic extracts of *A. auricula* 3 and *A. auricula* mix. There has not been found any results that would refer back to reducing sugar content of different strains of *A. auricula*. **Conclusion**

This research study was designed to determine approximate composition and antioxidant properties of *A. auricula*r strain 3, 5 and their mix. The result of this study reveals that *A. auricula* contains high quantity of fibre, protein and low level of lipid. *A. auricula* also contains many bioactive compounds with antioxidant property such as polyphenol, flavonoid, ascorbic acid and reducing sugars. The value of FRAP assay and DPPH free radical scavenging assay also suggest that *A. auricula* has high antioxidant property. Moreover, the present study also reveals that *A. auricula* 5 contain more nutritional and medicinal value than *A. auricula* 3. Based on the present investigations, we may conclude that *A. auricula* contain high nutritional and medicinal value. As it contain high antioxidant properties *A. auricula* can be used in different types of diseases like the prevention/treatment of cancer, tumor, viral diseases (influenza, polio), hypercholesterolemia, blood platelet aggregation, hypertension etc.

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