Development of *Agaricus bisporus* as a Nutraceutical of Tomorrow

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**Abstract**

*Agaricus bisporus* (AB), the most popularly consumed mushroom was chosen to be developed into a nutraceutical product. The important steps in the preparation of a nutraceutical i.e. standardization of processing method, its biological activity and safety index of the total extract were examined. The hexane, methanolic and aqueous (cold and hot) extracts of AB dried at optimized temperatures was tested for their action on free radicals in Di phenyl Picryl Hydrazyl (DPPH) assay. Finally, the safety of AB was established through in vivo tests up to a dose of 33 g/kg in mice.

**INTRODUCTION**

Any substance that may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease is known as nutraceutical (Committee on opportunities in the nutrition and food sciences, 1994). Chang and Buswell (1996) coined the term “mushroom nutriceuticals”, for the medicinal preparations from mushrooms and attempted to give a separate identity to this class of nutraceuticals. A mushroom nutriceutical is a refined/partially defined extract from either the mycelium or the fruiting body of the mushroom, which is consumed in the form of capsules/tablets as a dietary supplement and which has a potential therapeutic application. It is estimated that there are about 12,000 species of mushrooms of which about 50% are edible. However, of the 35 species that are commercially cultivated, only 7 to 8 are cultivated on an industrial scale (Chang, 1999). From a pool of six most widely used edible mushrooms, *Agaricus bisporus* (AB) was chosen for being developed into a “mushroom nutriceutical” based on market statistics.

**MATERIALS AND METHODS**

**Materials**

The mushrooms cultivated for commercial purposes under controlled conditions were procured from a local grower. A sample is preserved in the Natural Product department. Elisa plate reader (Multiskan, Labsystems, Helsinki, Finland), Microwave cooking systems (BPL 800T, 25 L capacity, Bangalore, India), Freeze drier (Heto dry winner, Model DW 1-0-110, Allerød, Denmark), Karl Fischer titrator (Metrohm, 703 Ti stand, herisau, Switzerland) were used in the experiments. Diphenyl picryl hydrazyl was procured from Sigma chemicals (Catalog no. D 9132, MO, USA) and the solvents were obtained from Ranbaxy (Delhi, India).

**Methods**

1. **Standardization of Drying Method.** 250 g mushroom were dried by lyophilization (pre-frozen at -40°C, with LSP 20 bars and HSP 25 bars), drying at physiological temperature of 37°C at RH 30-35% for 48 h or sun drying (38-41°C), RH 60-65% for 48 h, cooked in microwave (3 min) or retained fresh at room temperature (25-28°C). The sample retained at room temperature and the baked mushrooms were ground to a wet paste in the food processor and the rest of the samples were ground to a fine powder and passed through sieve # 10. The moisture content of the dried samples was determined through the Karl Fischer titrator.

2. **Extraction of Mushrooms.** The dried mushroom samples were powdered and...
sequentially extracted with hexane (1000 mL x 3), 70% methanol (1000 mL x 3) by maceration for 72 h, the solvent changed every 12 h with intermittent shaking. In case of hot aqueous extract, the marc after methanolic extraction was heated at 70 ± 5°C and filtered. The whole process was carried out at 10-12°C to yield a cold aqueous extract. The hexane (2.34 g) and methanolic extracts (5.62 g) were concentrated in vacuo and hot and cold aqueous extracts (6.23 and 4.78 g, respectively) were freeze-dried.

3. Estimation of Ergosterol. A HPLC method was developed using normal phase chromatography (hexane:ethyl acetate (8:2), flow rate of 0.5 mL/min, Nova Pak Silica column, dim 3.9 x 150 mm, PDA 996 Waters detector). Vitamin D$_2$ was detected at 262 nm and ergosterol was detected at 281 nm. The hexane extract (1 mg/mL) of the samples obtained by different methods of drying were quantified for the presence of ergosterol and vitamin D$_2$ content through the method of internal standardization.

4. Estimation of Carbohydrates. The carbohydrates were estimated by the Lowry method (Lowry et al., 1951).

5. Estimation of Proteins. The proteins were estimated by the Lowry method (Lowry et al., 1951).

6. DPPH Assay. Methanolic solution of DPPH (0.003%, 95 µL) was added to different dilutions of the sample (5 µL) in microtitre plates (Bouchet et al., 1998). The plates were incubated in dark conditions for 15 min and the absorption of the plates measured at 515 nm. Ascorbic acid was used as a standard. The IC$_{50}$ was calculated by Sigmaplot software version 8.0.

7. Safety and Toxicity. The no-observed-adverse-effect level (NOAEL - the highest intake of a nutrient at which no adverse effects have been observed) was determined by administering the total extract of AB dried at 37°C at an acute dose to mice (Swiss, male, body weight 30 g) in a half normal dose to reach 1000 mg per 30 g of mice through the oral route. The mice were observed for any adverse reaction or deaths.

8. Statistical Method. The IC$_{50}$ value was calculated by linear regression and values were expressed as Mean ± SEM.

RESULTS AND DISCUSSION

While removing the moisture content from AB (~ 85-95%) by various methods such as lyophilization, drying at optimal and high temperatures, it was observed that there is a variation in the content of carbohydrates (11.3 to 28.89%); proteins (15.51 to 23.33%) and ergosterol (40.04 to 90.35%) in the processed materials compared to that of its basal values found in unprocessed mushrooms (Table 1). The conversion of ergosterol to vitamin D$_2$ occurred only in samples processed at an optimum temperature of 35-38°C. However, all these nutrients were decomposed in the baked mushroom. From the results it is observed that drying at 37°C is most beneficial as it retains the maximum amount of its nutrient contents. From DPPH assay it can be proposed that the extracts exhibit mild antioxidant activity, the methanolic extract being the most active (Table 2).

The mushrooms are processed commercially by a number of methods depending upon the capacity and facility available. There is no standardized procedure used for the same and hence, the effect of drying needs to be assessed and standardized such that there is no major deterioration to nutraceutically important constituents like carbohydrates, proteins and vitamins. In case of vitamins, vitamin D$_2$ was evaluated for change in its contents, since ergosterol, the cell wall component of the mushroom is the biosynthetic precursor of vitamin D$_2$ (Nishino et al., 1980).

Free radical scavenging property, which may along with other activities contribute to the claimed therapeutic properties of AB. In the present study, DPPH assay was employed as a measure of antioxidant activity. DPPH is a colored free radical and the degree of discoloration indicates the direct free radical scavenging activity of substances (Bouchet et al., 1998; Viturro et al., 1999). The extract is capable of scavenging some types of reactive oxygen species and the total extract of AB, administered through oral route did not cause any lethality to mice up to a dose of 33 g/kg body weight of mice.
Literature Cited

Tables

Table 1. Nutrient contents of mushroom (*Agaricus bisporus*) dried by different methods. Values represent Mean ± SEM of % values obtained from three dilutions of samples.

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Moisture content (%)</th>
<th>Carbohydrate content (%)</th>
<th>Protein content (%)</th>
<th>Ergosterol content (%)</th>
<th>Vitamin D2 content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze dried</td>
<td>7.49</td>
<td>19.00 ± 0.42</td>
<td>21.07 ± 0.18</td>
<td>90.35 ± 0.07</td>
<td>NP*</td>
</tr>
<tr>
<td>Sun dried</td>
<td>3.33</td>
<td>11.37 ± 0.38</td>
<td>23.33 ± 0.43</td>
<td>40.04 ± 0.35</td>
<td>NP</td>
</tr>
<tr>
<td>Dried at 37°C</td>
<td>2.69</td>
<td>28.89 ± 0.82</td>
<td>15.51 ± 0.75</td>
<td>74.81 ± 0.68</td>
<td>31.37±1.02</td>
</tr>
<tr>
<td>Fresh</td>
<td>89.33</td>
<td>33.00 ± 1.02</td>
<td>15.80 ± 0.21</td>
<td>18.49 ± 0.42</td>
<td>NP</td>
</tr>
<tr>
<td>Microwave</td>
<td>87.82</td>
<td>2.97 ± 0.03</td>
<td>4.17 ± 0.13</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

* NP: Not Present

Table 2. Antioxidant activity of mushroom (*Agaricus bisporus*) observed using di-phenyl picryl hydrazyl (DPPH) assay. Values expressed as IC_{50} ± SEM represent means of three dilutions of samples.

<table>
<thead>
<tr>
<th>Extract</th>
<th>IC_{50} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>NP</td>
</tr>
<tr>
<td>Methanolic</td>
<td>145.55 ± 5.51</td>
</tr>
<tr>
<td>Cold aqueous</td>
<td>257.00 ± 1.44</td>
</tr>
<tr>
<td>Hot aqueous</td>
<td>288.57 ± 10.38</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>40.01 ± 4.09</td>
</tr>
</tbody>
</table>

* NP: Not Present