INTRODUCTION

*Armillaria mellea* Quel. is commonly called as Honey fungus or Bootlace fungus which belongs to the Kingdom- Fungi, Division- Basidiomycota, Class- Homobasidiomycetes, Order- Agaricales, family- Tricholomataceae, Genus- *Armillaria*, Species- *mellea*. *Armillaria* species are present in most natural and exotic forests, in orchards and parks, throughout the world, from the north temperate coniferous forests to the tropical forests and into the southern hemisphere. The genus includes at least thirty six species (Singer, 1978; Watling *et al.*, 1991; Volk and Burdsall, 1995), with seven morphological species present in Europe (Guillaumin *et al.*, 1985; Termorshuizen and Arnold, 1987). Some representatives of this genus are amongst the most significant root pathogens of trees and shrubs (Shaw and Kile, 1991), growing in clusters, causes serious sustained damage in certain regions, especially after several years of consecutive attack on the host (Guillaumin *et al.*, 1993). Rhizomorphs, thickened strands of mycelium, which aid in the spread of the fungus. Some species of *A. mellea* are bioluminescent and may be responsible for the phenomena known as foxfire and will o’ the wisp.

The nutritional value and sensory properties of edible mushroom is determined by their chemical composition. Varying opinions have been expressed regarding the true nutritive value of edible mushrooms. Since earliest times, mushrooms have been treated as a special kind of food. Mushrooms may be consumed for their palatability and or nutritional value. Palatability can be judge by colour, texture, flavour and taste, but the determination of nutritional value requires much scientific work. It involves the analysis of the proximate composition and a study of the spectrum of amino and fatty acids, vitamins, minerals and melic acid present. Mushroom is good source of protein and amino acid apart from vitamins and minerals. Its protein contains varies between 19-40% on dry weight basis.
protein contains most of the amino acids (Kutzman, 1975). They are rich in lysine and tryptophan but deficient in sulphur containing amino acid. It has been estimated (Maw and Flegg, 1974) that *Agaricus bisexualus* and *Volvarialla diplasia* contain higher amounts of proteins than several vegetables (viz. peas, cabbage, carrot, cauliflower, potato, tomato) and fruits (viz. apple, banana) but lower than some of the cereals (viz. rice, wheat) and animal products (viz. beef, egg, chicken, fish and cow milk). Analysis of data revealed that biological values of mushrooms proteins are intermediate between vegetables and animal proteins (Worgan, 1968).

Oxygen radicals and other reactive species derived from them are generated in biological systems either as by products of oxygen reduction or by xenobiotic catabolism (Chance et al., 1979). These active oxygen molecules are involved in infectious diseases in local infections and in inflammatory disease (Bast and Goris, 1989) and exert various deleterious effects in cells and tissues that basically depend on the subcellular structure where they are generated (Sies, 1985). When these species are generated within the hydrophobic domains of cell membranes, the free radical chain reaction of lipid peroxidation predominates. Lipid peroxidization products, e.g., phospholipids and fatty acid hydroperoxides, aldehydes and ketons contribute to the toxic effects exerted in hydrophobic domains (Chance et al., 1979; Bast and Goris, 1989; Sies, 1985). It has been observed that DNA is also a major target of oxidative injury (Ames, 1989). Being of particular importance in a large number of disorders such as cancer (Emerit and Cerutti, 1981) and degenerative diseases including Alzheimer’s disease, Parkinson’s disease and Hodgkin’s disease (Jenner, 1991). It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation, and may reduce potential mutations and, therefore, help prevent cancer or heart disease. Hence, it is of importance to find antioxidants in natural resources (Nakano, 1997; Tsushida et al., 1994; Nakatani, 1993).
In 1979 Ignaro made the initial discovery that bubbling NO gas into Krebs bicarbonate solution bathing isolated preconcentrated strips of coronary artery caused a marked relaxation response (Gruetter et al., 1979). This studies were completed prior to the discovery of endothelium-derived relaxing factor (EDRF) (Furchgott and Zawadzki, 1980). They have also shown that NO act as a potent inhibitor of platelet aggregation and that NO elucidated such affects by stimulatory platelet cyclic GMP formation (Mellion et al., 1981). Research on NO increased dramatically during the late 1980s NO was then identified as an important cytotoxic protein of cytokine activated macrophage (Hibbs et al., 1988; Marletta et al., 1988). A series of interesting studies lead to the appreciation that NO plays some neurotransmission role in the central nervous system (CNS). It is now clear that NO has numerous role in biological system which includes: vasodialation (Ignaro, 1996), regulation of blood pressure (Fledman et al., 1993), signal molecule in smooth muscle and nerve (Kahn et al., 2000), inhibit platelet aggregation and adhesion (McDonald et al. 1993), inhibitor of neutrophil adhesion (Ignaro, 1996), a neuromodulator in the CNS (Ignaro, 1996), antioxidant (Beckman, 1996), antithrombotic (Sinha et al., 1998) and second messenger of insulin (Kahn et al., 2000).

The basidiomycete fungus A. mellea, was reported to produce in culture, compounds having anti-bacterial and antifungal activities (Donnelly et al., 1985). A. mellea is also possess significant potential against the poliomyelitis virus in mice (Cochran, 1978). A. mellea is reported to be an excellent purgative, medicine for stimulating the secretion of bile, medicine for dizziness and headache (Gareth, 1990). In the Chinese market, the crude form of the drug, which contains extracts of its artificially cultured mycelium, is sold to treat geriatric patients with palsy, dizziness, headache, neurasthenia, insomnia, numbness of the limbs, and infantile convulsions. The basidiomes of A. mellea contain the medicinal substances like armillaramide, a new sphingolipid (ceramide) obtained by fractionating the
ethyl acetate extract of sporophores. Sphingolipids are important building blocks of the plasma membrane of eukaryotic cells. Some of these substances have been recently reported to exhibit antihepatotoxic, anti-tumour and immunostimulatory activities (Gao et al., 2001). *A. mellea* is an important medicinal fungi symbiotically associated with Tianma a traditional Chinese medicine (*Gastrodia elata* Blume).

The present chapter includes the studies on the pharmacognostic, nutritional, antioxidant and nitric oxide synthase activation properties of *A. mellea*. 
MATERIALS AND METHODS

Materials and methods followed in this chapter have been discussed previously.

RESULTS AND DISCUSSION

Macroscopic characters of *Armillaria mellea* Quel.

Sporophores solitary, gregarious or sometimes singly on hardwoods or on the forest floor, appearing soon after the first fall rains, honey coloured and known as Honey or Bootlace mushroom (Figure 35A). Pileus 3.0-15.0 cm broad, spherical at first, then becoming convex, later expanded and subumbonate, margin finely striate, glabrous, colour varying to honey to dull reddish brown, dark coloured at the centre, occasionally with small scales sometimes scales entirely absent. Gills distinctly formed, subdistant, adnate or decurrent, whitish when young, becomes coloured when age, 0.5-1.2 cm broad developing reddish-brown strains in age. Stipe central, uniform in thickness, 2.0-17.0 cm long, 1.2-3.0 cm thick, tapering towards the base when growing in clusters, enlarged to bulbous at the base when growing apart. Stipe is generally yellowish or brownish, whitish at the top, elastic, stuffed or hollow, stipe annulated, ring prominent, well developed, white, thick, not movable. Flesh white, firm, thick gradually thinner towards the margin. Surface vicid when moist. Odor mild, taste acrid.

Microscopic characterization of *Armillaria mellea* Quel.

Hymenophoral trama bilateral. Basidium was elongated club shaped bearing four long tube like structure at the apex known as sterigmata. Each sterigmata bears single basidiospore. Basidiospores white, smooth, rounded or elliptical, nonamyloid, 7.6-10.0 x 5.0-6.3 µm. Spore print white, marginal cystidia present (Figure 35B, 35C).
Analyses of powdered material.

The powdered fruit body is dark brown in colour with a pleasant aromatic smell and characteristic taste. The microscopic examination of powdered material reveals the presence of spores and numerous distorted mycelium of different sizes (Figure 35D, 35E). The size, shape and colour of the spores were similar to those of the fresh fruit body, which may help to identify the material.

Powder studies:

Powder when treated with different chemical reagents showed different colour reactions tabulated in table 31 and 32.

Table 31: Behaviour of powdered sample on treatment with different chemical reagents.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Colour observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder as such</td>
<td>Reddish black</td>
</tr>
<tr>
<td>with Picric acid (saturated aqueous solution)</td>
<td>Snuff brown</td>
</tr>
<tr>
<td>with concentrated HNO₃</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>with concentrated HCl</td>
<td>Umber</td>
</tr>
<tr>
<td>with H₂SO₄ (80%)</td>
<td>Date brown</td>
</tr>
<tr>
<td>with CH₃COOH (Glacial)</td>
<td>Lemon yellow</td>
</tr>
<tr>
<td>with FeCl₃ (5% aqueous solution)</td>
<td>Brownish black</td>
</tr>
<tr>
<td>with NaOH (5 N aqueous solution)</td>
<td>Blackish red</td>
</tr>
<tr>
<td>with I₂ (aqueous solution)</td>
<td>Blackish brown</td>
</tr>
</tbody>
</table>
Table 32: *Observation of fluorescence characters of powdered material under UV light.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder as such</td>
<td>Black</td>
</tr>
<tr>
<td>Powder mounted in nitrocellulose paper</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH in water</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH in water, dried and mounted with nitrocellulose</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH with methanol</td>
<td>Fluorescence green</td>
</tr>
<tr>
<td>Powder treated with 1(N) HCl</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with HNO₃ dilute with equal volume of water</td>
<td>Yellow</td>
</tr>
<tr>
<td>Powder treated with 50% HCl, dried and mounted with nitrocellulose</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder treated with H₂SO₄ dilute with equal volume of H₂O</td>
<td>Light green</td>
</tr>
</tbody>
</table>

The behavior of the dry powdered of *Armillaria mellea* Quel. on treatment with different chemical reagents showed different types of colour reactions (Table 31) and also specific fluorescent characteristic when subjected under ultraviolet light (Table 32). All these characteristics will help in proper identification of the specimen as a whole and its powder for future studies.

**Physico-chemical studies:**

A known quantity of dried powdered material was extracted in a Soxhlet apparatus with petroleum ether, Benzene, chloroform, acetone, methanol, ethanol and water successively. The percentage of each extractive was calculated by evaporation of the respective solvents (Figure 36). The results showed that the water and chloroform shows maximum (17%) and minimum (0.28%) extractive values respectively.
The colour of different extractives showed significant differences (Table 33).

**Table 33: Colour of extractives.**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Colour of extractives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>Creamish white</td>
</tr>
<tr>
<td>Benzene</td>
<td>Yellowish</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Faint yellow</td>
</tr>
<tr>
<td>Acetone</td>
<td>Faint cream</td>
</tr>
<tr>
<td>Methanol</td>
<td>Faint yellow</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>

**Figure 36:** Successive extractive values of *Armillaria mellea* Quel. Results are the mean of three separate experiments, each in triplicate.
On preliminary phytochemical examination of different extracts the presence of alkaloids, polyphenols, sterols, carbohydrate and flavonoids were detected (Table 34).

### Table 34. Preliminary phytochemical tests for presence of active constituents:

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloid</th>
<th>Polyphenols</th>
<th>Sterols</th>
<th>Carbohydrate</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive  
-  = negative

Unlike higher plants, from the dry powdered materials of mushrooms it was very difficult to identify the purity of the material by microscopic study because it consists of deformed mycelium. So the above mentioned qualitative and quantitative pharmacognostic parameters may play the crucial role for identification of the purity of a dry powdered mushroom sample.

**Nutritional composition of Armillaria mellea Quel.**

The mushroom protein is known to contain almost all the essential amino acids. The quantitative spectrum of essential amino acids has served as the basis to calculate biological value, nutritional value and protein score (Zeisel, 1999). The protein content of *A. mellea* Quel. was estimated by following Lowry’s method and found to be 24.47 ± 1.35 g (n=3) / 100 g of dry tissue (Figure 37). According to Hayes (1975) the mushroom protein has a high digestibility and in its overall quality can be classed as being intermediate between low grade vegetable and high grade meat protein.
Mushroom contain large amounts of carbohydrates including polysaccharides such as glucans and glycogen, monosaccharides and disaccharides (such as trehalose), sugar alcohols (such as manitol) and chitin. Most of the polysaccharides are structural components of the cell walls, chitin and glucans and are indigestible by humans; thus they may be considered as dietary fiber. Dietary fiber was declared a nutrient by Nutrition Labeling and Education Act of 1993 (Gordon, 2002). The soluble carbohydrate and total content of this mushroom was estimated by Dinitrosalicylic acid (DNSa) method and was found to be $28.68 \pm 3.66 \text{ g}$, $60.08 \text{ g} \pm 3.23 \text{ g (n=3)}$ / 100 g of the dry tissue respectively.

The crude fibre content of the sample was estimated by following the method of Maynard (1970) and found to be $15.8 \pm 0.2 \text{ g (n=3)}$ / 100 g of dry tissue (Figure 37). Fiber is now considered to be an important ingredient in a balance and health diet (Chang and Miles, 1989). The free amino acid content of the fungus was extracted and quantified following the procedure of Sadasivam and Manickam (1996). The quantitative amount of free amino acids present in the sample was found to be $11.08 \pm 2.9 \text{ g (n = 3)}$ of dry thallus (Figure 37).

The fat content of the mushrooms is low, ranging from 1.1 to 8.3% on dry weight basis with an average content of 4.0% (Chang and Miles, 1989). Total fat content of the mushroom was isolated and extracted by following the method of Folch et.al. (1991) and was estimated to be $2.8 \pm 0.85 \text{ g (n= 3)}$ / 100 g of dry tissue (Figure 37).
Moisture content of *A. mellea* was 92.26% of the fresh material and the ash content was found to be 19.9 g / 100 g of dry thallus (n= 3).

The fruitbody of mushrooms are characterised by a high level of well assailable mineral constituents (Breen, 1990; Vetter, 1994; Demirbas, 2001; Falandysz *et al.*, 2001; Mattila *et al.*, 2001). Results in the Figure 38 showed values of macro and micro mineral compositions. The mineral contents were: calcium 40 mg / 100 g dry wt, potassium 940 mg / 100 g dry wt, magnesium 130 mg / 100 g dry wt, phosphorous 1730 mg / 100 g dry wt and iron 25 mg / 100 g dry wt.

**Figure 37: The proximate composition of *A. mellea* Quel.** Results are the mean ± SD of three separate experiments, each in triplicate.
Figure 38: Mineral composition of *A. mellea* Quel. Results are the mean of three separate experiments, each in triplicate.

Analysis of proximate composition of *A. mellea* Quel. revealed that this mushroom is rich in protein and carbohydrate, moderate in crude fibre, low in fat content which could serve a proper diet for the sufferers of hypertension, high blood pressure, atherosclerosis etc. This mushroom is a good source of essential amino acids and minerals. Phosphorus and potassium are the two dominant elements in mineral portions. Considering all these values this mushroom is a low caloric, crude fiber rich which can be used as a safe diet for sufferers of several killer diseases. Furthermore, due to the presence of high level of crude fiber it could help to control diabetes and obesity.

**In vitro antioxidant properties of different extracts of *A. mellea* Quel.**

Free radicals react with biological molecules and destroy the structure of induced disease such as cancer, renal failure, ageing etc. reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxides and lipid peroxide as are generated by several oxidative reactions (Vuillaumeone, 1987). Although ROS can help the immune system to clean out extensive
microorganism, excessive ROS can also react with biological molecules such as DNA protein and phospholipids, and eventually causes oxidative damage in tissues and free radical-related diseases such as inflammation, heart diseases, diabetes, cancer etc (Slater, 1984). Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The hydroxyl radical (OH·) thus produced may attack the sugar of DNA base causing sugar fragmentation, base loss and DNA strand breakage (Kaneko et al., 1996). Crude, boiled and ethanolic extracts of A. mellea Quel. showed significant scavenging activity of OH· radical. IC₅₀ value for crude, boiled and ethanolic extracts were 388.92 ± 78.25, 219.48 ± 48.36, and 36.3 ± 4.75 µg / ml, respectively (Figure 39). These extracts possessed significantly higher activity than catechin (840 ± 25 µg / ml).

![Figure 39: Hydroxyl radical scavenging activity of different extracts of Armillaria mellea. Quel Results are the mean ± SD of three separate experiments, each in triplicate.](image)

Oxidation of lipid membrane in the biological system is very destructive process associated with liver injury, carcinogenesis and the aging process. In
this study, *in vitro* lipid peroxidation was induced to red blood cells by using ferrous sulphate and ascorbic acid. Crude, boiled and ethanolic extracts of *A. mellea* Quel. also showed significant inhibition lipid peroxidation activity. IC\textsubscript{50} values for crude, boiled, and ethanolic extracts were 101.7 ± 20.4, 198 ± 31.2 and 93.3 ± 5.84 µg / ml (Figure 40). Crude and ethanolic extracts possessed significantly higher activity than catechin (455 ± 32 µg/ml). However, the boiled extract showed slightly higher activity than catechin.

![Figure 40: Inhibition of lipid peroxidation by extracts of Armillaria mellea. Quel. Results are the mean ± SD of three separate experiments, each in triplicate.](image)

DPPH, a special free radical with characteristics absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to their radicals, the absorbance decreases. The decrease in absorption was taken as a measure of the extent of the radical scavenging. The IC\textsubscript{50} values of the crude, boiled and ethanolic extracts were found to be 106.25 ± 14.9, 92.1 ± 11.03 and 107.07 ± 17.73 µg / ml respectively, values were significant when compared to synthetic antioxidant standard drug BHT (85.7± 3.4 µg / ml) (Figure 41).
The results of the investigations revealed that all the extracts of *A. mellea* Quel. had potent DPPH, hydroxyl radical scavenging and lipid peroxidation inhibition activities. The results indicated that extracts of *A. mellea* Quel. possessed significant antioxidant activity thus suggested the therapeutic value of this mushroom.

**In vitro NOS activation properties of different extracts of *A. mellea* Quel.**

Nitric oxide recognized to be an inter - and intra – cellular mediator of several cell function and it acts as a signal molecule in immune, nervous and vascular systems (Schmidt *et al.*, 1993). All the three extracts i.e. crude, boiled and ethanolic extract of *A. mellea* Quel. showed significant increase in nitric oxide production over control due to the activation of NOS (Figure 42). The results were 188.31 ± 18.6, 104.6 ± 23.1, 711.46 ± 61.1 pmol / mg dry wt / h respectively. Use of 10 µM N° methyl – L – arginine acetate ester (NAME), a competitive inhibitor of nitric oxide synthase (NOS) (Sprague *et al.*, 1994), in the reaction mixture showed complete inhibition of NO production in all cases, indicating the increased production of NO was due to the activation of
NOS. Ethanolic extracts showed a very significant increase in NOS activity, indicating the therapeutic importance of this mushroom.

![Graph showing nitric oxide production by different extracts of Armillaria mellea Quel over the control.](image)

**Figure 42**: *Production of nitric oxide by different extracts of Armillaria mellea Quel over the control*. Values are the mean ± SD of three separate experiments each in triplicate.

*A. mellea* Quel. is an excellent, edible mushroom used as delicacies. Antioxidant activity and NOS activation potentiality of the edible mushroom has significant importance because their activity greatly contributes to their nutraceuticals properties, thus enhancing their nutritive value and lessening our demand for the therapeutic chemicals in use at present for the treatment of different ailments, thus mushroom nutraceutical as well as mushroom as a functional food are likely to be an increasing interest throughout the world (Saini and Atri, 1999).
REFERENCES


