INTRODUCTION

*Auricularia auricula* (Hook.) Underw or jelly mushroom is commonly called as ‘jew’s ear’, ‘wood ear’ or ‘tree ear’ (Wasser and Weise, 1999). In Darjeeling it is called as ‘kanay chaw’. *Auricularia auricula* (Hook.) Underw belongs to the Kingdom·Fungi, Division·Basidiomycetes, Class·Homobasidiomycetes, Order·Auriculariales, Family·Auricularaceae, Genus·*Auricularia* and Species·*auricula*. They are facultative parasites growing on trunks of many broad leaf trees or on dead wood (Hobbs, 1995). They have worldwide distribution, both temperate and tropical parts of the world, widespread in United States, Europe and Asia. *Auricularia auricula-judae* was first intentional cultivated edible and medicinal mushroom goes back to 600 A.D (Chang and Miles, 2004). Historically it has been used in China both as food and medicine.

The chemical composition of edible mushroom determines their nutritional value and sensory properties. Mushrooms have been used as a food supplement in various culture and they are cultivated and eaten for their edibility and delicacy. They fall between the best vegetables and animal protein source. Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001; Buigut, 2002). Mushrooms are good source of vitamins like riboflavin, biotin and thiamin (Chang and Buswell, 1996). Ogundana and Fagade (1981) indicated that in mushroom there is about 16.5% dry weight, of which 7.4% crude fiber, 14.6% crude protein and 4.48% fat and oil. The protein value of mushroom is twice as that of *Asperagus* and potatoes, four times as that of tomatoes and carrots and six times as that of oranges (Jiskani, 2001).

Potentially harmful reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism. The reactive species is usually inactivated *in vivo* by a variety of antioxidants. Antioxidants are deployed to prevent generation of ROS or to scavenge those formed. Thus, oxidatively induced tissue damage is minimized. However, deficiency of antioxidant
defenses may lead to oxidative stress, which might be associated with a variety of disorders, arthritis, and cancer (Yoshikawa et al., 2000; Spiteller, 2001). When natural defenses are overwhelmed by excessive generation of pro-oxidants, a situation of oxidative stress evolves, and cellular macromolecules might suffer oxidative damage (Sies, 1996; Weisman and Halliwell, 1996). A number of methods have been developed to measure the efficiency of antioxidants. These methods focus on different mechanisms of the antioxidant defense system such as scavenging or inhibiting free radicals or chelation of metal ions that otherwise may lead to free radical formation. These free radicals are capable of inducing damage to all cellular molecules, which can lead to diseased states (Yoshikawa et al., 2000; Spiteller, 2001). Synthetic antioxidants also have been developed to retard lipid peroxidation, but their use as food supplements and therapeutic agents have been hindered due to their possible toxicity.

Nitric oxide (NO) which is produced in mammalian system by an enzyme nitric oxide synthase (NOS) was first identified as endothelium derived relaxing factor (EDRF) (Furchgott and Zawadzki, 1980) and identified from vascular endothelium cells. 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), 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).

_Auricularia_ has been used for thousands of years for the treatment of various diseases. It is prepared in a different way for each different group of treatments. For example, hypertension, vascular sclerosis and ophthalmic bleeding are all in one group and rheumatic pains in legs and lumbago are in another. _Auricularia_ has been used over the centuries, to treat weakness after childbirth, cramp and numbness; for pains from injury or sound,
obstruction in arteries and veins, numbness and tetany; for malignant
dysentery, piles, and enteritis; for menorrhagia and leucorrhoea; for gastric
disorder causing nausea and excessive phlegm; for piles in the aged which
will not heal (Ying et al., 1987). There are reports on its effectiveness in
reducing the blood glucose levels in KK·Ay mice that are genetically obese
and have diabetic syndromes such as, hyperglycemia, hyperinsulinemia,
gluco-suria and severe insulin resistance, hypocholesteromic activity (Yuan et
al., 1998a; 1998b: Saini and Atri, 1999). The water soluble polysaccharide
possess anti-tumor activity (inhibition of sarcoma 180 cells) (Ceruti et
al.,1993; Ukai et al.,1983), produce a hypocholesterolemic effect (Ryong and
Tertov, 1989), inhibit liver lipid accumulation, reveal antioxidant properties
and explicate a hypoglycemic effect on genetically diabetic mice (Stamets,
2000). Extracts of Auricularia prevent egg implantation in animals
terminating early and mid· pregnancy (Ho and Chen, 1991). In Auricularia
polytricha, an anti· platelet substance (Adenosine) has been reported to have
the capability to inhibit platelet aggregation (Markhija and Bailly, 1981).
Auricularia is said to have been used in folk medicine in Hong Kong to thin
the blood and reduce clotting problems in post· partum women and also they
have been traditionally used for treating hemorrhoids and various stomach
ailments (Chang and Buswell, 1996).
The present chapter deals with the pharmacognostic, nutritional, antioxidant
and nitric oxide synthase activation properties of Auricularia auricula
(Hook.) Underw.
MATERIALS AND METHODS

Materials and methods were discussed inditail in earlier section of this thesis.

RESULTS AND DISCUSSION

Macroscopic characters of *Auricularia auricula* (Hook.) Underw.

Sporophores growing solitary in dense tufts on dead wood and logs or on tree trunks. Carpophore or fruiting body (Figure 3A) usually 6-10 cm, ear shaped, jelly like or gelatinous, usually sessile or with a short attaching peduncle, shallow cup shaped or flattened, outer surface sterile, pubescent, with slight venation, inner surface fertile, yellow brown to reddish brown when moist and flexible, flesh soft, slightly elastic, translucent, fragile, becoming horney and brittle on drying, no particular odor or flavour.

Microscopic characterization of *Auricularia auricula* (Hook.) Underw.

The carpophore of the *Auricularia auricula* are differentiated into three layers, which can be distinguished into a thin and compact upper layer, generally tomentose with simple hair, an intermediate layer of thin gelatinous hyphae and a thin layer constituting the hymenium (Figure 3B). The basidium is phragmobasidium, divided into epibasidium and hypobasidium. Each cell of the basidium produces a long tube like structure at the apex known as sterigmata, this structure is formed on the tip. The basidium is elongated and elliptical subtending on the sterigmata. Spore white, cylindrical, smooth, 12-15 × 4-6 µm (Figure 3C).

Analyses of powdered material.

Certain physical characters like color, odour and taste of the powdered material were evaluated. The powdered fruit body is dark brown in colour with a pleasant aromatic smell and characteristic taste. On microscopic
examination it shows spores and numerous distorted mycelium of different size. The size, shape and colour of the spores were similar to that of the fresh fruit body, which may help to identify the material (Figure 3D, 3E).

**Powder studies:**

Powder when treated with different chemical reagents showed different colour reactions tabulated in table 15 and 16.

**Table 15. 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>Greyish brown</td>
</tr>
<tr>
<td>with Picric acid (Saturated aqueous solution)</td>
<td>Brownish yellow</td>
</tr>
<tr>
<td>with concentrated HNO₃</td>
<td>Brownish</td>
</tr>
<tr>
<td>with concentrated HCl</td>
<td>Brownish black</td>
</tr>
<tr>
<td>with H₂SO₄(80%)</td>
<td>Blackish grey</td>
</tr>
<tr>
<td>with CH₃COOH (Glacial)</td>
<td>Greyish brown</td>
</tr>
<tr>
<td>with FeCl₃(5% aqueous solution)</td>
<td>Blackish</td>
</tr>
<tr>
<td>with NaOH (5 N aqueous solution)</td>
<td>Greyish</td>
</tr>
<tr>
<td>with I₂ solution</td>
<td>Brownish black</td>
</tr>
</tbody>
</table>

**Table 16: 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>Grey</td>
</tr>
<tr>
<td>Powder mounted in nitrocellulose paper</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH in H₂O</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH in water, dried and mounted with nitrocellulose</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder treated with 1 (N) NaOH with methanol</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with 1 (N)HCl</td>
<td>Fluorescence green</td>
</tr>
<tr>
<td>Powder treated with HNO₃ diluted with equal vol. of wa</td>
<td>Fluorescence green</td>
</tr>
<tr>
<td>Powder treated with 50% HCl, dried and mounted with nitrocellulose</td>
<td>Greenish</td>
</tr>
<tr>
<td>Powder treated with H₂SO₄ dil. with equal vol. of H₂O</td>
<td>Fluorescence green</td>
</tr>
</tbody>
</table>
The behaviour of the dry powdered of *Auricularia auricula* (Hook.) Underw on treatment with different chemical reagents showed different types of colour reactions (Table 15) and also specific fluorescent characteristic when subjected under ultraviolet light (Table 16). 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 then water successively. The percentage of each extractive was calculated by evaporation of the respective solvents (Figure 4). The results showed that the water and chloroform shows maximum (15 %) and minimum (0.52%) extractive values respectively.

![Figure 4: Successive extractive values of *Auricularia auricula* (Hook.) Underw. Results are the mean of three separate experiments, each in triplicate.](image)

The colour of different extractives showed significant differences (Table 17).
Table 17. *Colour of extractives.*

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

On preliminary phytochemical examination of different extracts the presence of alkaloids, polyphenols, sterols, carbohydrate and flavonoids were detected (Table 18).

Table 18. *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</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

Now a day modern medicinal science looking for the development of new drugs against the killer diseases from the lower groups of plants and fungi including mushrooms. Food of fungal origin is consumed all over the world in vast quantities and commercial production is part of the rapidly growing industry (Ingram, 2002). 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 *Auricularia auricula* (Hook.) Underw.**

The mushroom protein is known to contain almost all the essential amino acids. The quantitative spectrum of essential amino acids have served as the basis to calculated biological value, nutritional value and protein score (Haque, 1989). The protein content of *A. auricula* (Hook.) Underw. was estimated by following the by Lowry’s method and found to be $19.8 \pm 2.7$ g (n = 3) / 100 g of dry tissue (Figure 5). The soluble and total carbohydrate content of this mushroom was estimated by dinitrosalicylic acid (DNSa) method, was found to be $29.06 \pm 3.17$ g, $53.5 \pm 4.7$ g (n = 3) / 100 g of the dry tissue respectively (Figure 5).

The crude fibre content of the sample was estimated by following the method of Maynard (1970) and found to be $15.9 \pm 1.6$ g (n = 3) / 100 g of dry tissue (Figure 5). Fiber is now considered to be an important ingredient in a balance and healthy 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 $6.01 \pm 1.78$ g (n = 3) / 100 g of dry thallus (Figure 5).

The content of the fats in mushroom is low ranges from 1.1 to 8.3% on dry weight basis with an average content 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 $2.7 \pm 0.76$ g (n= 3) / 100 g of dry tissue (Figure 5). The moisture content of *Auricularia auricula* was 94.7% of the fresh materials and the ash content was found to be $19.9$ g (n= 3) / 100 g of dry thallus.
The fruit body of mushrooms are characterised by a high level of well assimilability mineral constituents (Breen, 1990; Vetter, 1994; Demirbas, 2001; Falandysz et al., 2001; Mattila et al., 2001). Results in the Figure 6 showed values of macro and micro mineral compositions. The mineral contents were: calcium 250 mg / 100 g dry wt, potassium 1040 mg / 100 g dry wt, magnesium 80 mg / 100 g dry wt, phosphorous 725 mg / 100 g dry wt and iron 61 mg / 100 g dry wt.
Analysis of proximate composition of *Auricularia auricula* (Hook.) Underw reveal that this mushroom is rich in protein and carbohydrate, moderate in crude fibre, ash and low in fat content. 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.

**In vitro antioxidant properties of different extracts of A. auricula:**

Free radicals are chemical entities that can separately with one and more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals (Cotran *et al.*, 1999; Yu *et al.*, 1992). Antioxidant may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation etc. Ferrous salts can react with hydrogen peroxide and form hydroxyl radical via fenton’s reaction. The iron require for this reaction is obtained either from the pool of
iron or the heme-containing protein (Cotran et al., 1999). 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. auricula* showed significant scavenging activity of OH· radical. IC$_{50}$ value for crude, boiled and ethanolic extracts were 403 ± 35, 510 ± 66, and 373 ± 13 µg / ml, respectively (Figure 7). These extracts possessed significantly higher activity than catechin (840 ± 32 µg / ml).

![Figure 7](image_url)

**Figure 7:** *Hydroxyl radical scavenging activity of different extracts of *Auricularia auricula* (Hook.) Underw.* Results are the mean ± SD of three separate experiments, each in triplicate.

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver (Coyle and Puttfarcken, 1993). 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. auricula* also showed significant inhibition lipid peroxidation activity. IC$_{50}$ values for crude, boiled, and ethanolic extracts were 310 ± 8.1, 572 ± 24.6 and 398 ± 16.8 µg / ml.
(Figure 8). Crude and ethanolic extracts possessed significantly higher activity than catechin (455 ± 25µg / ml). However, the boiled extract showed slightly higher activity than catechin.

![Figure 8: Inhibition of lipid peroxidation by extracts of Auricularia auricula (Hook) Underw. Results are the mean ± SD of three separate experiments, each in triplicate.](image)

Superodixes are produced from molecular oxygen due to oxidative enzyme of the body as well as via non enzymatic reactions such as auto-oxidation of catecholamines (Hemmani, and Parihar, 1998). In the present study superoxide radicals were generated from the auto-oxidation of hematoxilin and was detected by an increased in absorbance at 560 nm (Martin et al., 1987). The superoxide radical scavenging activity of the crude (47 µg / ml), boiled (80 µg / ml) and ethanolic (50 µg / ml) extracts of A. auricula showed the maximum inhibition of 16.7, 5.56, and 18.8 % respectively. Reason for the low superoxide scavenging activity of extracts was unknown. However, herbs that scavenge superoxide contain a component of flavonoids which are widely distributed in plants (Josh and Janardhanan, 2000).
DPPH is a relatively stable free radical and the assay determines the ability of different extract to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to the paired ones. Antioxidant can act by converting the unpaired electrons to paired one. IC_{50} values of crude, boiled and ethanolic extracts were 129.33 ± 14.9, 93.58 ± 11.03 and 127.79 ± 17.73 μg /ml respectively. These values were significant when compared to synthetic antioxidant standard drug BHT (85.7 ± 3.4 μg / ml) (Figure 9).

![Figure 9: Free radical scavenging capacity of different extracts of Auricularia auricula (Hook.) Underw measured in DPPH assay. Results are the mean ± SD of three separate experiments, each in triplicate.](image)

The results of the investigations revealed that all the extracts of *A. auricula* had potent DPPH, hydroxyl radical scavenging and lipid peroxidation inhibition activities. The results indicated that extracts of *A. auricula* possessed significant antioxidant activity thus suggested the therapeutic value of this mushroom.
**In vitro NOS activation properties of different extracts of A. auricula**

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). Further study was made to evaluate the nitric oxide synthase activation properties of crude, boiled and ethanolic extracts of *A. auricula*. All the three extracts i.e. crude, boiled and ethanolic extract of *A. auricula* showed significant increase in nitric oxide production over control (Figure 10), these were 644 ± 25, 191 ± 20, 850 ± 45 pmol / mg dry wt / h respectively. Use of 10 µM N^G^ 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. Ethanol 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 A. auricula](image)

**Figure 10:** Production of nitric oxide by different extracts of *Auricularia auricula* over the control. Values are the mean ± SD of three separate experiments each in triplicate.
*A. auricula* is an excellent and delicious edible mushroom, hence the possibilities of cytotoxicity of the extract of the mushroom cannot envisage. Using the proximate analyses and the mineral values as approximate indices in nutritional quality, it would appear that this mushroom fall between most legumes and meat. This mushroom is known to possess hypocholesteromic activity, which implies that mushroom could hold special attraction for and may be recommended for cholesterol related ailments (Yuan *et al.*, 1998a; 1998b; Saini and Atri, 1999). Because of the high level of crude fiber this mushroom can be used a dietary supplement for the people suffering from diabetes and obesity. The results of the nutritionally valuable minerals showed that this mushroom species was rich in calcium, potassium, magnesium, iron and phosphorus. Minerals in the diet are required for metabolic reaction, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others. From the study, it is observed that this edible mushroom holds tremendous promise in complementing the protein and mineral supply deficit prevalent in developing countries. The result of the present investigation showed valuable therapeutic use of this mushroom that can be used for the prevention and control of several diseases.
REFERENCES


Studies on pharmacognostic, nutritional, antioxidant and nitric oxide synthase activation properties of some wild edible mushroom of Darjeeling Himalaya


