Antioxidant and nitric oxide synthase activation properties of *Auricularia auricula*

Krishnendu Acharya*, Krishnendu Samui, Manjula Rai, Bani Brata Dutta & Rupa Acharya

Biotechnology Research Laboratory,
Post Graduate Department of Botany, Darjeeling Government College, Darjeeling 734 101, India

Received 10 September 2003; revised 23 December 2003

In vitro evaluation of antioxidant activities of *Auricularia auricula* showed significant inhibition of lipid peroxidation, and potently scavenge free radicals when compared with standard drug catechin. IC50 value of crude, boiled and ethanol extracts of *A. auricula* represented 403, 510, and 373 µg/ml respectively in case of hydroxyl radical scavenging activity and 310, 572 and 398 µg/ml respectively in case of lipid peroxidation. Furthermore, crude, boiled and ethanol extracts also increase significantly nitric oxide production (664, 191 and 850 pmole/mg dry wt/hr respectively) over the control. The present results revealed that *A. auricula* had potential therapeutic use.

**Keywords:** Antioxidant activity, *Auricularia auricula*, Nitric oxide synthase

**IPC Code:** Int. Cl. A61B

Mushrooms are nutritionally functional food and a source of physiologically beneficial and non-toxic medicine. They have been used as folk medicine throughout the world since ancient time. Besides being a healthy food, mushrooms are used as ailments for the persons suffering from cancer, heart ailments, diabetes, high blood pressure, constipation, renal failure etc.

*Auricularia auricula* (Hook.) Underw, belongs to the family Auriculaceae, is an edible mushroom, commonly called tree-ear or wood-ear. They have worldwide distribution, both temperate and tropical parts of the world.

Earlier reports have shown that polysaccharides of *Auricularia* sp have stimulatory effects on the immune system of human. In some cases it causes the production of interferon and interleukins that stop the proliferation of cancer cells, they have also been found to have anti-tumour, cardiovascular and hypcholesterolemia, anti-viral, anti-bacterial and anti-parasitic effects.

Reactive oxygen species (ROS) are produced continuously in the cells as accidental by-products of metabolism which are cytotoxic agents important factors for several pathological conditions such as cardiovascular diseases, diabetes, inflammation, cancer etc. Antioxidants act as a major defense against radical-mediated toxicity by protecting the damages caused by free radicals.

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) and identified from vascular endothelium. It is now clear that NO has numerous roles in biological systems including vasodilation, regulation of blood pressure, inhibit platelet aggregation and adhesion, inhibitor of neutrophil adhesion, a neuromodulator in the CNS, antioxidant, antithrombotic and second messenger of insulin.

The present study was conducted to evaluate the antioxidant activity and NOS activation properties of the different extracts of *A. auricula*.

**Collection of materials**—Basidiocarp of *Auricularia auricula*, commonly called as kaney chau by the local people Kanie Chau, was collected from local market and from the forest of Darjeeling, India, and identified according to Ramsbottom (1965) and Bessey (1978).

**Extraction**—Crude extracts were prepared from fresh tissues (1 g/10 ml of distilled water) after homogenization in 0.1 M phosphate buffer, (pH-7.4) and centrifugation at 15,000 x g for 30 min at 4°C. Supernatant stored at 0°C for further use. Boiled extracts were prepared from fresh fruit body (1 g/10 ml of distilled water) after boiling it in water bath for 1 hr, then homogenized and centrifuged 15,000 x g for 30 min at room temperature and finally supernatant was taken for further use. Dry powdered fruit body (50 g) was extracted with 750 ml of ethanol (95%) in a soxhlet apparatus for 20 hr. The ethanolic extract was subjected to distillation under reduced pressure. A brownish material was obtained and stored in refrigerator.

**Assay of hydroxyl radical**—Hydroxyl radical (OH) was generated from Fe2+-ascorbate- EDTA-
H₂O₂ system (Fenton’s reaction) which attack the deoxyribose and set of a series of reaction that eventually resulted in formation of MDA, measured as a pink MDA-TBA chromogen at 532 nm. Reaction mixture (1 ml) contained deoxyribose (2.8 mM), KH₂PO₄-KOH (20 mM; pH 7.4), FeCl₃ (100 mM), EDTA (104 μM), H₂O₂ (1 mM) and ascorbate (100 μM). Reaction mixture was incubated at 37°C for 1 hr and colour developed as described above. IC₅₀ value of deoxyribose degradation by the crude, boiled and ethanolic extracts A. auricula over the control were measured, catechin was used as a positive control.

**Assay of lipid peroxidation**—Lipid peroxidation was induced by Fe²⁺-ascorbate system in human red blood cells (RBC) and estimated as thiobarbituric acid reacting substances (TBARS) by the method of Buege and Aust (1978). The reaction mixture contained RBC- packed cell (10⁸ cell/ml) in Tris-HCl buffer (20 mM, pH 7.0) with CuCl₂ (2 mM), ascorbic acid (10 mM) and different extracts of A. auricula in final volume of 1 ml. The reaction mixture was incubated at 37°C for 1 hr. Lipid peroxidation was measured as malondialdehyde (MDA) equivalent using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375% w/v, TBA: 15% w/v TCA; and 0.25% HCl). The incubated reaction mixture was mixed with 2 ml of TBA-TCA reagent and heated in a boiling water bath for 15 min. After cooling the flocculants precipitate was removed by centrifugation at 1000 x g for 10 min. Finally malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. The crude, boiled and ethanolic extracts concentrations, that would inhibit by 50% the production of thiobarbituric acid-reactive substances, i.e., IC₅₀ value were calculated. Catechin was used as a control.

**Assay of superoxide**—Superoxide radical (O₂⁻) was generated from auto-oxidation of hematoxilin and was detected by an increase in absorbance at 560 nm, in a Beckman DU6 spectrophotometer. The reaction mixture contains 0.1 M of phosphate buffer (pH-7.4), EDTA (0.1 mM), hematoxilin (50 μM) and incubated at 25°C for different time periods. Inhibition of auto-oxidation of hematoxilin by crude, boiled and ethanolic extracts over the control were measured.

**Determination of nitric oxide (NO) synthase activity**—NO was determined according to Jia et al. by using scanning Beckman spectrophotometer (Model DU6). Typically, NO content of the supernatant was determined by the conversion of oxyhemoglobin to methemoglobin. The reaction mixture containing RBC (10⁸ cells) was incubated with L-arginine (10 μM); haemoglobin (64 mM) with different concentrations of crude, boiled and ethanolic extracts of A. auricula; in a total volume of 2.5 ml for different time periods at 37°C. After each incubation period, a portion of reaction mixture was centrifuged at 8,000 x g for 5 min at 37°C and NO content of the supernatant was compared with an appropriate control set.

**Statistical analysis**—Results were subjected to statistical analysis using Student’s t test. Values are mean ± SD of 3 replications. Results are significant P<0.001 vs Standard.

Crude, boiled and ethanolic extracts of A. auricula showed significant scavenging activity of OH radical generated from Fe²⁺-ascorbate-EDTA - H₂O₂ system. IC₅₀ value for crude, boiled and ethanolic extracts were 403, 510, and 373 μg/ml, respectively. These extracts possessed significantly higher activity than catechin (840 μg/ml). Crude, boiled and ethanolic extracts of A. auricula also showed significant inhibition lipid peroxidation activity. IC₅₀ values for crude, boiled, and ethanolic extracts were 310, 572 and 398 μg/ml. Crude and ethanolic extracts possessed significantly higher activity than catechin (455 μg/ml). However, the boiled extract showed slightly higher activity than catechin. The super oxide radical scavenging activity of the crude (47 μg/ml), boiled (80 μg/ml) 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 contains a component of flavonoids which are widely distributed in plants. The present results revealed that all the extracts of A. auricula had potent 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.

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. 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 of A. auricula showed significant increase in nitric oxide production over control (Fig. 1), these were 644 ± 25, 191 ± 20, 850 ± 45 pmole/mg dry wt/hr respectively. Use of 10 μM of NG methyl-L-arginine acetate ester (NAME), a competitive inhibitor of nitric oxide synthase (NOS)19, in the reaction mixture showed complete inhibition of NO production in all cases, indicating the increased production of NO in present study was due to the activation of NOS. Ethanolic extracts showed a very significant increase in NOS activity, indicating the therapeutic importance of this mushroom.

A. auricula is a delicious edible mushroom, hence the possibilities of cytotoxicity of the extract of the mushroom cannot envisaged. Our earlier investigation has indicated that A. auricula is a nutritionally functional food. 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