8

Macrofungi – Bioactive Potential

8.1 Introduction

In the interest of human health, exploration of natural products gained utmost importance compared to synthetic drugs. Although fungi are the diverse dominant group of life forms, a fraction of them are described (~100,000) and a few species are explored for bioactive metabolites (De Silva et al., 2013). However, success of many fungal-derived secondary metabolites (e.g. antibiotics, cholesterol-lowering agents, immunosuppressive drugs and mycotoxins) projects their future potential. Mushrooms are believed to be an important natural source of remedial products for a variety of human ailments (Abraham, 2001; Aly et al., 2011; Wasser, 2011). Asian countries are historically depending on indigenous macrofungi for several centuries to treat many of human diseases (e.g. Ying et al., 1987; Aly et al., 2011; Xu et al., 2011). A variety of potential compounds possessing useful biological activities (e.g. antibiotics, antiviral, cytotoxic and pharmacological) were isolated from macrofungi (e.g. Bao et al., 2001; Zhang et al., 2007; Jeong et al., 2011; De Silva et al., 2013). Many edible mushrooms besides serving as potential nutritional value, they are known to serve as nutraceuticals through diet-management in prevention of cardiovascular diseases, hypocholesterolemia and atherosclerosis due to their high fiber and low fat content (De Silva et al., 2013). Western Ghats of India is endowed with a variety of macrofungi of nutritional, medicinal and industrial importance (Thatoi and Singhdevsachan, 2014; Mohanan, 2011; Farook et al., 2013). Although they are traditionally used for nutrition and medicinal purposes, a precise picture on their importance is yet to emerge. Therefore, the present study attempts to evaluate two wild edible mushrooms in the Western Ghats and west coast of India to link their bioactive components with functional attributes (antioxidant properties). As they
are edible, dry flours of uncooked as well as cooked mushrooms are evaluated for their potent components and antioxidant potential.

8.2 Materials and methods

8.2.1 Mushrooms and processing

*Auricularia auricula-judae* (Bull.) Quél. (Auriculariaceae-Basidiomycotina) and *Termitomyces umkowaan* (Cooke & Massee) D.A. Reid (Lyophyllaceae-Basidiomycotina) were collected from Kadnur, Virajpet of the Western Ghats (12°13'N, 75°46'E; 891 m asl) and from mixed forest of Mangalore University Campus, Mangalore, west coast (12°48'N, 74°55'E; 112.4 m asl), respectively during July-August, 2013. They were processed as detailed in Section 6.2 for assessment of bioactive potential.

8.2.2 Bioactive principles

*Total phenolics*

Total phenolics of mushroom samples was determined based on the method outlined by Rosset *et al.* (1982). Mushroom flours of 50 mg each were extracted in 5 ml methanol (50%) in water bath (95±1°C) for 10 min followed by centrifugation (1500 rpm) and collection of supernatant. Methanol extraction was repeated once again for the flour pellet and pooled supernatant was made up to 10 ml. Aliquots of 0.5 ml extract was mixed with 0.5 ml distilled water, mixed with 5 ml Na$_2$CO$_3$ (in 0.1 N NaOH) and incubated for 10 min at laboratory temperature. Folin-Ciocalteu’s reagent 0.5 ml (diluted, 1:2 v/v with distilled water) was added and absorbance was read at 725 nm (UV-VIS Spectrophotometer-118, SYSTRONICS, Ahmedabad, Gujarat, India) with tannic acid as standard. The results were expressed in mg of tannic acid equivalents per gram of the sample (mg TAEs/g).

*Tannins*

To assess tannins in mushroom flours, vanillin-HCl method by Burns (1971) was used. Mushroom flour (1 g) was extracted with methanol (50 ml) at 28°C up to 24 hr, followed by centrifugation (1,500 rpm) and collection of supernatant. Aliquots of 1 ml supernatant was treated with 5 ml vanillin hydrochloride reagent (mixture of 4% vanillin in methanol and 8% concentrated HCl in methanol; ratio, 1:1). On incubation up to 20 min the developed color was read at 500 nm and catechin (98% HPLC grade, Sigma Aldrich, USA)
served as standard. The results were expressed in mg of catechin equivalents per gram of the sample (mg CEs/g).

**Flavonoids**

Content of flavonoids in mushroom flours was evaluated by following the procedure by Chang *et al.* (2002). Mushroom flours were extracted in methanol at a concentration of 1 mg/ml. Aliquot of 0.5 ml methanolic extract was mixed with methanol (1.5 ml), aluminium chloride (10%, 0.1 ml), potassium acetate (1M, 0.1 ml) and distilled water (2.8 ml). Absorbance was measured at 415 nm after incubation for 30 min at laboratory temperature. Quercetin served as standard and the results were expressed in mg quercetin equivalents per gram sample (mg QEs/g).

**Vitamin C**

Content of vitamin C of mushroom flours was determined based on Roe (1954) with a slight modification. One gram of mushroom sample was extracted with trichloroacetic acid (TCA: 5%, 10 ml). Aliquot of 0.2 ml was made up to 1 ml with TCA (5%) and 2,4-dinitrophenylhydrazine (DNPH) (1 ml) was added. This reaction mixture was boiled up to 10 min, cooled to laboratory temperature, sulfuric acid was added (65%, 4 ml) and incubated up to 30 min at laboratory temperature followed by measurement of absorbance at 540 nm with ascorbic acid as standard. Vitamin C content was expressed as ascorbic acid equivalents in mg/g of mushroom flour (mg AAEs/g).

**L-DOPA**

The L-DOPA (L-3,4-Dihydroxyphenylalanine) was determined according to the protocol by Fugii *et al.* (1991) (Fig. 8.1). Aliquot of mushroom powder was mixed with 1 ml distilled water followed by incubation up to 2 hr at laboratory temperature. After centrifugation (1,500 rpm), supernatant was concentrated using rotary evaporator to dryness. The extract was dissolved in distilled water, filtered (Ultrafilter: TOYO ROSHI KAISHA Ltd., Japan) and kept for overnight to eliminate compounds of higher molecular weight. The low molecular weight fraction was further purified using a ODS mini column (C18 Sep-Pak Cartridge, Waters) with 100% water. After concentrating the extract to dryness, L-DOPA was analyzed by HPLC and LC-ESI/MS.
Fig. 8.1. Protocol employed for extraction of L-DOPA from *Auricularia* and *Termitomyces* using HPLC and LC–MS (DW, distilled water).
Trypsin inhibition

To determine trypsin inhibition activity of mushroom flours, enzymatic assay proposed by Kakade et al. (1974) was employed. One gram mushroom flour was extracted with NaOH (0.01 N, 50 ml), one ml of the extract was made up to 2 ml using distilled water. Two ml of trypsin solution (4 mg in 200 ml 0.001 M HCl) was added followed by incubation in a water bath (37°C) up to 10 min. To each tube, 5 ml of BAPNA [(40 mg N-a-Benzoyl-DL-Arginine p-nitroanilide hydrochloride in 1 ml dimethyl sulfoxide diluted to 100 ml with tris-buffer at 37°C)] was added. On incubation up to 10 min, the reaction was terminated by adding 1 ml acetic acid (30%). After thorough mixing, it was filtered and absorbance was measured at 410 nm against reagent blank (1 ml, 30% acetic acid containing 2 ml each of trypsin and distilled water + 5 ml BAPNA). One unit of trypsin inhibition (TIu/mg) is defined as release 1 μM of p-nitroanilide per min by the enzyme. 

Hemagglutination

The hemagglutinin activity of mushroom flours was evaluated according to the method by Occena et al. (2007). Mushroom flour (500 mg) was suspended in NaCl (10 ml, 0.9%) followed by vigorous shaking, allowed to stand for 1 hr and centrifuged (2,000 g, 10 min) to obtain clear solution. It was filtered and the filtrate was used as crude agglutinin extract. The RBCs were separated from the whole human blood suspension (5 ml) (A, B, AB and O groups) followed by centrifugation (2,000 g, 10 min). The RBCs were diluted with cold saline (0.9%) (1:4), centrifuged (2,000 g, 10 min) and the supernatant was eliminated. The RBC pellet was washed using saline until the supernatant became colorless. Four ml of washed erythrocytes were suspended in 100 ml phosphate buffer (0.0006 M, pH 8.4). One ml trypsin solution (2%) was added to 10 ml washed erythrocytes, mixed and incubated at 37°C up to 1 hr. The trypsinized erythrocytes were washed (4–5 times in saline) to remove traces of trypsin. The packed cells (1.2–1.5 ml) were suspended in 100 ml saline.

Microtitre plates (8 rows of 12 wells) were used to assess hemagglutinin activity of mushroom flours. In the first well the crude agglutinin extract was added and the well 12 served as control as it has no crude agglutinin extract. The saline (0.3 ml) was dispensed to well # 2 to 12. Serial dilution was followed from the well # 2 to 11. Trypsinized RBC (in saline 2%, 0.3 ml) was dispensed to wells # 1 to 12. The contents were mixed and incubated at laboratory temperature up to 4 hr. The pattern of hemagglutination in each well was noted and the hemagglutinating unit per gram (Hu/g) was determined by the following formula:
\[
Hu/g = (D_a \times D_b \times S) - V
\]

(1)

(where \(D_a\), dilution factor of extract in well # 1 is the crude agglutinin extract it remains as 1 if the original extract is not diluted; \(D_b\), dilution factor of well containing 1 Hu is the well in which hemagglutination is first seen; \(S\), ml original extract/g mushroom flour; \(V\), volume of extract in well # 1).

8.2.3 Antioxidant Assay

Antioxidant capacity of test sample is influenced by various factors and demands at least two methods have to be adapted for assessment (Wong et al., 2006). Thus, in the present study four assay methods were employed to evaluate antioxidant properties of mushroom samples: i) Reduction of Mo(VI) to Mo(V) by antioxidant compounds (total antioxidant activity, TAA); ii) Reduction of Fe(III) to Fe(II) ions (Fe\(^{2+}\) ion chelating capacity); iii) DPPH radical absorption on exposure to radical scavengers (radical-scavenging activity); iv) Conversion of Fe\(^{3+}/\)ferricyanide complex into the ferrous form (reducing power).

Methanol extract of mushroom flour was prepared by extracting 0.5 g flour in 30 ml methanol (shaken at 150 rpm for 48 hr). After incubation, the extract was centrifuged and supernatant was collected in a pre-weighed Petri dish and allowed to dry at laboratory temperature. The weight of the extract was determined gravimetrically and dissolved in methanol to get the desired concentration (1 mg/ml) to perform antioxidant assays.

**Total antioxidant activity**

For total antioxidant activity (TAA), extract (0.1 ml) was mixed with 1 ml reagent mixture (sulphuric acid, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4 mM) (Prieto et al., 1999). It was incubated at 95°C up to 90 min, cooled and absorbance of phosphomolybdenum complex was measured at 695 nm with methanol blank. The TAA was expressed as \(\mu\)M equivalent of ascorbic acid per gram of the mushroom flour (mg AAEs/g).

**Ferrous-ion chelating capacity**

Method outlined by Hsu et al., (2003) was employed to determine Fe\(^{2+}\) chelating capacity of mushroom samples. To one ml of extract, FeCl\(_2\) (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) were added followed by making up to 5 ml in methanol. After incubation up to 10 min at laboratory temperature absorbance of Fe\(^{2+}\)-ferrozine-complex was determined at
562 nm. The sample devoid of extract served as control to calculate ferrous ion chelating capacity:

\[
\text{Ferrous ion chelating capacity (\%) } = 1 - \left( \frac{A_{c562} - A_{s562}}{A_{c562}} \right) \times 100
\]

(2)

(2)

\[\text{where } A_c, \text{ absorbance of the control; } A_s, \text{ absorbance of sample}\]

**DPPH free radical-scavenging activity**

Free radical-scavenging activity of mushroom extracts was evaluated based on the method by Singh *et al.*, (2002). Concentrations ranging from 200-1000 µg (0.2-1 ml) of test sample was made up to 1 ml in methanol, 4 ml 2,2-diphenyl-1-picrylhydrazyl (DPPH; 0.01 mM) was added and allowed to react at room temperature up to 20 min. Reagents devoid of extract served as control, the absorbance of mixture was measured at 517 nm and free radical-scavenging activity was calculated:

\[
\text{Free radical-scavenging activity (\%) } = \left[ \frac{(A_{c517} - A_{s517})}{(A_{c517})} \right] \times 100
\]

(3)

(3)

\[\text{where } A_c, \text{ absorbance of the control; } A_s, \text{ is absorbance of sample}\]

Effective concentration (EC_{50}; concentration of sample required to scavenge 50% of DPPH radicals) (µg extract/ml) was obtained on plotting per cent radical-scavenging activity against concentration of the extracts.

**Reducing power**

Reducing power of the extract was determined according to the method by Pulido *et al.* (2000) with minor modification. Concentrations ranging from 200–1000 µg (0.2–1 ml) of mushroom flour extracted in methanol were mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). After mixing the contents it was incubated at 50°C up to 20 min, 2.5 ml of TCA (10%) was added, centrifuged (3,000 rpm) up to 10 min and 2.5 ml supernatant was mixed with 2.5 ml distilled water. Ferric chloride (0.1%, 0.5 ml) was added to the mixture followed by measurement of absorbance at 700 nm. Increase in absorbance of the reaction mixture indicates increase in reducing power.

**8.2.4 Data analysis**

Difference in bioactive components between uncooked and cooked mushroom flours was assessed by *t*-test using Statistica version # 8.0 (StatSoft Inc., 2008).
8.3 Results and discussion

As in foods of plant origin, extensive interest has been developed to investigate bioactive potential of wild and cultivated mushrooms. Therapeutic potential of many macrofungi growing in wild or in cultivation is still not clearly understood although they are consumed and used for medicinal purposes. Considerable studies have been performed especially in the East Asian countries to employ many macrofungi for therapeutic purposes as they are traditionally used (Kalač, 2009). Nutritional and health-promoting/disease-resistance (or nutraceutical) power of macrofungi is more valuable than the synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ). Synthetic antioxidants used widely in food industry have been reported to be carcinogenic and their use needs to be restricted (Botterweck et al., 2000).

Total phenolics of many macrofungi serve as major antioxidants as well as free radical-scavengers. The quantity of total phenolics was substantially lower in *A. auricula* than in *T. umkowaan* without significant change on cooking (Fig. 8.2). In uncooked samples of *T. umkowaan* total phenolics was high and decreased significantly to one-third on cooking. Total phenolics of *T. umkowaan* is higher than many termitomycetes (*T. badius*, *T. medius*, *T. radicatus* and *T. striatus*) (21.4 vs. 15–20.1 mg/g), comparable with *T. heimii* (21.3 mg/g) (Kumari, 2012) and lower than *T. mammiformis* and *T. microcarpus* (21.4 vs. 22.5–37 mg/g) (Kumari, 2012). Interestingly, although very high quantities of total phenolics were reported in *T. mammiformis* and *T. robustus* (211.8 and 178 mg/g, respectively) from Nigeria, their antioxidant potential was not considerably high (Unekwa et al., 2014).

Tannin content was higher in *T. umkowaan* than in *A. auricula* with significant decrease on cooking (Fig. 8.2). However, its content in mushrooms of the same genera was higher as reported by Abdullah et al. (2012) (*A. auricula*: 0.37 vs. 6.2 mg/g; *T. umkowaan* vs. *T. heimii*: 0.37 vs. 11.3 mg/g). The tannin content reported in other termitomycetes in Nigeria was substantially high (*T. mammiformis* and *T. robustus*: 169.2 and 170.6 mg/g, respectively), but their antioxidant activity was not higher than other mushrooms (Unekwa et al., 2014).

Flavonoids consist of several biologically active compounds like flavones, isoflavones, flavonols, flavanols and anthocyanins possessing anti-diabetic, anti-inflammatory, hepatoprotective, anti-thrombotic, anti-atherosclerotic, anti-neoplastic and cardioprotective properties (Champ 2002; Tapas et al. 2008). Although flavonoids are nutritionally important, their reports in mushrooms are sporadic (Barros et al., 2008; Gursoy
Fig. 8.2. Total phenolics (TAEs, tannic acid equivalents), tannins (CEs, catechin equivalents) and flavonoids (QEs, quercetin equivalents) in uncooked and cooked *Auricularia* and *Termitomyces* (n=5, mean±SD) (different letters on the bars represent significant difference: *, p<0.05; **, p<0.01; t-test).
et al., 2009). Flavonoids were higher in *A. auricula* than in *T. umkowaan* with significant decrease up to 50% on cooking, while cooking has no significant change in the latter (Fig. 8.2). The flavonoids in uncooked *A. auricula* of Nigeria is extremely low compared to the uncooked as well as cooked *A. auricula* in the present study (6.4 vs. 9.4–21.6 mg/g) (Unekwa et al., 2014). Flavonoids of uncooked *T. umkowaan* is higher than other termitomycetes (*T. badius, T. heimii, T. medius, T. mammiformis, T. microcarpus, T. radicatus and T. striatus*) (4–4.1 vs. 1.4–2 mg/g) (Kumari, 2012). Flavonoids were as high as 23.9 and 25.7 mg/g in termitomycetes in Nigeria (*T. robustus* and *T. mammiformis*) (Unekwa et al., 2014).

Natural products rich in total phenolics have the capacity to decrease the incidence of atherosclerosis, cancer and coronary heart diseases (Randhir et al., 2004; Alothaman et al., 2009). In the present study, uncooked *T. umkowaan* consists of high quantities of total phenolics, tannins and flavonoids, so also the flavonoids in uncooked *A. auricula*. Decrease in phenolics and tannins in these mushrooms on cooking can be attributed to leaching as well as formation of complexes with proteins on pressure cooking. The results on total phenolics clearly reveal such a change occurred only in *T. umkowaan* than in *A. auricula* possibly due to higher quantity of total proteins (18.9–21.5 vs. 6.1–6.4%) (see Table 7.1). In uncooked *A. auricula*, total phenolics was low and its quantity did not significantly varied on cooking may be due to low protein content (6.1–6.4%) (see Table 7.1). Unlike total phenolics, cooking resulted in significant drastic decrease in tannins of both mushrooms.

Although vitamin C serves as a potent antioxidant, pro-oxidant and radical scavenger, its loss takes place due to thermal treatment of foodstuffs (Podmore et al., 1998; Gregory, 1996). Vitamin C was higher in *A. auricula* than in *T. umkowaan* without significant change on cooking (Fig. 8.3). The vitamin C content in uncooked *T. umkowaan* was lower than other termitomycetes (*T. heimii, T. mammiformis, T. radicatus and T. reticulatus*) (0.12 vs. 0.24–1.45 mg/g). Interestingly, even though pressure cooking drastically reduced the vitamin C content in *T. umkowaan*, it was not significantly decreased in *A. auricula* as seen in total phenolics.

The L-DOPA is biologically inactive non-protein amino acid useful in treatment of Parkinson’s disease (Hornykiewicz, 2002). Its content was substantially higher in *T. umkowaan* compared to *A. auricula* and decreased substantially in both mushrooms on cooking (Fig. 8.3).

According to Acharya et al. (2004), *A. auricula* has very high potential of inhibition of lipid peroxidation as well as hydroxyl radical-scavenging ability. In *A. auricula* of Nigeria,
Fig. 8.3. Vitamin C (AAEs, ascorbic acid equivalents) and L-DOPA in uncooked and cooked Auricularia and Termitomyces (n=5, mean±SD) (different letters on the bars represent significant difference: *, p<0.05; **, p<0.001; t-test).
the total phenolics, tannins and flavonoids were high (116, 66.9 and 6.4 mg/g, respectively), but the antioxidant activity was not much elevated (Unckwa et al., 2014). Purified watersoluble polysaccharide obtained from A. auricula showed potent antioxidant activity in vivo in mice model and in turn serve as valuable agent for anti-aging therapy (Zhang et al., 2011). Similarly, water-soluble β-D-glucan isolated from A. auricula serve as potent anti-tumor agent by inducing apoptosis against Sarcoma-180 solid tumor based on in vitro and in vivo anti-tumor assays (Ma et al., 2010). The mycelial methanol extract of T. albuminosus showed high antioxidant activity, reducing power and radical-scavenging potential (Mau et al., 2004). Evaluation of 23 species of indigenous species of mushrooms (encompassing five species from the Western Ghat forests of Kerala) showed good nutraceutical properties (Puttaraju et al., 2006). In particular, Termitomyces heimii and T. mummiformis showed maximum antioxidant potential with presence of high content of active principles like tannic acid, gallic acid, protocatechuic acid, and gentisic acid.

The total antioxidant activity was higher in A. auricula than in T. umkowaan without significant decrease on cooking, while it decreased significantly about 50% in cooked T. umkowaan (Fig. 8.4). It is likely the polysaccharides of A. auricula might be responsible for higher total antioxidant activity in uncooked as well as cooked samples as reported by Zhang et al. (2011). Metal-ion chelating capacity becomes important because such ions cause lipid peroxidation leading to food deterioration and in turn causing arthritis and cancer (Gordon, 1990; Halliwell et al., 1995). In this study, ferric-ion chelating activity was higher in T. umkowaan than in A. auricula with significant decrease on cooking (Fig. 8.4). The DPPH radical-scavenging assay helps to evaluate the capacity of bioactive components in mushrooms to serve as radical-scavengers especially the hydrogen donors. Radical-scavenging activity was higher in A. auricula than in T. umkowaan with significant decrease on cooking only in A. auricula. The reducing power was also higher in uncooked than in cooked mushrooms, which significantly decreased on cooking (Fig. 8.5). The reducing power of uncooked A. auricula was higher than the report by Abdullah et al. (2012) (1 mg/ml, absorbance at 700 nm: 0.207 vs. 0.110) (Fig. 8.5).

In spite of low total phenolics and tannins, uncooked as well as cooked A. auricula showed higher total antioxidant activity, DPPH radical-scavenging capacity and reducing power than T. umkowaan. It is likely the quantities of flavonoids, vitamin C and L-DOPA in A. auricula could be responsible for such results. Similarly, high quantities of total phenolics, tannins, vitamin C and L-DOPA especially in uncooked T. umkowaan might be responsible
Fig. 8.4. Total antioxidant activity and Fe$^{2+}$ chelating capacity (600 µg/ml) in uncooked and cooked *Auricularia* and *Termitomyces* (n=5, mean±SD) (different letters on the bars represent significant difference: *, p<0.05; **, p<0.01; t-test).
Fig. 8.5. The DPPH radical-scavenging activity (1 mg/ml) and reducing power in uncooked and cooked Auricularia and Termitomyces (n=5, mean±SD) (different letters on the bars or lines represent significant difference: *, p<0.05; **, p<0.01 (t-test).
for good total antioxidant activity as well as ferrous-ion chelating capacity. Interestingly, although total phenolics, tannins, flavonoids, vitamin C and L-DOPA contents were lower in cooked than in uncooked *T. umkowaan*, the ferric-ion chelating capacity as well as the DPPH radical-scavenging activity were considerably high in cooked samples denotes the possibilities of involvement of other bioactive principles not affected by pressure-cooking.

Uncooked and cooked samples of both mushrooms did not show trypsin inhibition activity (Table 8.1), which is nutritionally advantageous. In addition, the hemagglutination activity was substantially low (4–16 Hu/g) in both mushrooms (Table 8.1) qualify them as better source of nutrition than those mushrooms possessing high hemagglutination activity. There was no change in hemagglutination of blood group A+ve in uncooked and cooked mushrooms (16 Hu/g), so also in *A. auricula* and *T. umkowaan* against AB+ve and B+ve blood groups (8 Hu/g), respectively. Decrease in hemagglutination was seen between uncooked and cooked *A. auricula* against O+ve (8 vs. 4 Hu/g), *T. umkowaan* against AB+ve (16 vs. 8 Hu/g) and O+ve (16 vs. 4 Hu/g) blood groups. However, *A. auricula* showed increased hemagglutination activity on cooking against B+ve blood group (8 vs. 16 Hu/g).

Now-a-days dyslipidemia is responsible for antioxidant stress and atherosclerosis, which can be combated using functional diet developed by blending polysaccharide derived from *A. auricula* with processed Hawthorn fruits (*Crataegus*) (4:1%) (Luo et al., 2009). It showed increased radical-scavenging, inhibition of low density lipoprotein-cholesterol oxidation, lowered serum total cholesterol and low atherogenic index. Besides, such functional formulations showed several additional pharmaceutical advantages (Luo et al., 2011). Microwave-assisted extraction of polysaccharides from *A. auricula* (showed no influence on their structure and molecular weight) possess remarkable *in vitro* antioxidant activity and safe to use in food products based on toxicological evaluation (Zeng et al., 2012).

The products derived from uncooked *A. auricula* and *T. umkowaan* will be more beneficial than cooked ones. Besides, the nutritional qualities (e.g. proximal features and minerals) will be reduced in pressure-cooked mushrooms (see Section 6). Besides medicinal uses, there are several innovative applications of wild mushrooms for health benefits especially in formulation of functional foods. For example, blending polysaccharide flour derived from *A. auricula* at 9% with bread did not alter the nutritional and sensory qualities, which resulted in marked increase in antioxidant property (DPPH radical-scavenging) (Fan et al., 2006). Thus, alternate methods of cooking (e.g. partial conventional/microwave cooking) should be applied to retain maximum quantity of bioactive compounds as well as nutritional
Table 8.1. Trypsin inhibition and hemagglutinin activity of uncooked and cooked mushrooms (based on three independent observations).

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<tr>
<th></th>
<th><em>Auricularia auricula-judae</em></th>
<th><em>Termitomyces umkowaan</em></th>
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<td></td>
<td>uncooked</td>
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<td>Trypsin inhibition activity</td>
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<td>Hemagglutinin activity (Hu/g)</td>
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NP, Not Present
qualities to derive maximum multifunctional nutraceutical benefits from these traditional wild mushrooms. Further, it is necessary to test the relevance of these mushrooms as antioxidant agents in vivo with appropriate markers. Besides the bioactive components evaluated in the present study, the antioxidant potential of *A. auricula* and *T. umkowaan* might have been influenced by other potential components like stilbenes, lignans, phytoates, amino acids, peptides, vitamin E, carotinoids, specific fatty acids and specific minerals needs further precise investigation.

### 8.4 References


