# CHAPTER-2

# Nutritional and bioactive potentials of selected mushrooms in Gurguripal Ecoforest

## 2.1. Introduction

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. From ancient period, wild mushrooms have been consumed by people with delicacy for their pleasing flavour and taste (Das 2010). Many genera of mushrooms are considered to be edible, rich in essential nutrients such as proteins, carbohydrates, minerals, vitamins, fibres and essential amino acids (Okwulehie and Odunze 2004). Apart from their nutritional attributes, mushroom are also natural sources of physiologically valuable bioactive substances that promote our health (Kumari et al. 2017). Some of mushroom species are consumed as nutraceuticals (natural product having potentiality in boosting health and immune system of human) while others as potent nutraceuticals (compounds that have medicinal as well as nutritional values and are consumed in the form of capsules or tablets) (Elmastas et al. 2007; Ribeiro et al. 2007). Mushrooms have profound nutritional and medicinal usefulness towards human health and development (Rai et al. 2005 and Wani et al. 2010).

Besides nutritional attributes, many mushrooms are acknowledged for their antioxidant and other biological potentials, thereby serving as source of nutraceuticals (Barros et al. 2007a; Ferreira et al. 2009; Heleno et al. 2015). Imbalanced metabolism causes oxidative stress by an excess production of reactive oxygen species (ROS) in our body creates a range of disorders like metabolic diseases, heart diseases and severe neurological disorders. ROS are not only generated internally in the body but also through the influence of various external sources such as UV light, ionizing radiations, chemotherapeutics and

environmental toxins. Inhaling toxic substances from surroundings has become unavoidable in modern civilization. Though the presence of oxidative defence mechanism in the body, sometimes it becomes insufficient to control excessive oxidative damage. In this situation the intake of antioxidants from external sources becomes essential, where mushroom showed enormous significance (Mitra et al. 2016). Moreover, antioxidants are extensively used in food packaging, health care, anti-aging and cosmetics. The global business for antioxidants is growing fast and has grown more than double from $103.6 million in 2011 to reach $246.1 million in 2018 (Antioxidants Market—Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2014–2020). The market of antioxidants by product type was categorised into natural antioxidants and synthetic antioxidants. Among the synthetic phenolic antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and others such as propyl gallate, ethoxyquin (EQ), *tert*-butylhydroquinone (TBHQ) can effectively inhibit oxidative stress (Venkatesh and Sood 2011). However, many synthetic antioxidants cause adverse effects under certain conditions in our body (Ferreira et al. 2009; Kozarski et al. 2014). BHA, which is very commonly used as an additive in food industry, at high concentrations have negative impacts on the regulation of the activity of mitogen-activated protein kinase (MAPK) (Yu et al. 1997). In recent past, the restriction on the use of synthetic antioxidants like BHA and BHT, has enhanced the interest towards searching of natural antioxidant substances. In this prospect, edible mushrooms have attracted a lot of scientific attention as a natural source of antioxidants (Ferreira et al. 2009; Khatua et al. 2013; Kozarski et al. 2014). They might be consumed directly in the enhancement of antioxidant defence system through dietary supplementation to reduce the level of excess oxidative stress. Mushrooms have the ability to accumulate some metabolites such as terpenes and steroids, phenolic compounds, polyketides (Turkoglu et al. 2007). These metabolites influence odour, taste, appearance and oxidative stability of nutrients in

mushrooms (Singh et al. 2012). The antioxidative constituents found in mushrooms are majorly phenolic compounds reported to have protective role against persistent diseases related to oxidative stress (Ferreira et al. 2009). Previously, Mallavadhani et al. (2006) has opined that the bioactivity of phenolics is related to their ability of chelating metals, inhibiting lipoxygenase and scavenging free radicals.

Not only phenolic compounds, but also mushrooms possess fatty acids, which are important constituents of fungal cells with recognized roles as components of plasmalemma, cell organelle membranes and as storage material. In fungi, the major fatty acids are palmitic and stearic acids, and their unsaturated derivatives like palmitoleic, oleic, linoleic, linolenic acids etc. typically constitute the membrane phospholipids and storage triacylglycerols (Suutari 1995). Mushrooms possess highly valuable fatty acids, among them palmitic, oleic and linoleic acids are found as the most abundant fatty acids found in members of Basidiomycetes. Pedneault et al. (2006) has reported that, linoleic and linolenic acids are essential for nutrition and metabolism in humans, while long-chain polyunsaturated fatty acids (PUFA) have many beneficial effects on human health.

In Asia mushrooms are used as natural source of medication to protect human body from various diseases elicited by oxidative stress (Chen et al. 2012). In developing countries like India, mushrooms have an immense scope for progress in the field of food, medicine and generating employment. The tribal people of Gurguripal lives in concord with nature and for a major portion of resources of their livelihood they are heavily dependent on the forest. Particularly wild mushrooms serve a vital food source in their nutritional requirements.

The present chapter deals with the estimation of proximate composition, fatty acid analysis and antioxidant activity of polyphenol rich fraction of selected mushroom species consumed by local people living in and around Gurguripal ecoforest.

## 2.2. Materials and Methods

### *2.2.1. Analysis of nutrients*

Among the 67 mushroom species occurring in Gurguripal ecoforest 9 species were found as predominant on the basis of edibility (Section 1.3.1). In order to assess the health benefits of those 9 prime edible mushroom species the present study was designed to analyse their proximate composition. During the present investigation the mushroom species, namely *Agaricus* sp., *Amanita bisporigera*, *Astraeus hygrometricus*, *Cantharellus* sp., *Termitomyces medius*, *Pleurotus ostreatus*, *Schizophyllum commune*, *Termitomyces heimii and Volvariella volvacea* were evaluated for their nutritional components following standard biochemical techniques.

*2.2.1.1. Preparation of sample for analysis*

The whole fruit bodies of mushroom species were cleaned properly and finely chopped into pieces. Then the pieces were dried for 4-5 hours at 52-57 ℃ in a food dehydrator (Presto, USA). Next the dried mushroom pieces were collected and subjected to grinding (KenStar, India). The fine mushroom powder (sample) was poured into zipped polythene bags and kept under vacuum to avoid moisture. This procedure was followed for all nine mushroom species separately.

*2.2.1.2. Determination of total protein*

About 5 g of sample was dissolved with 50 ml of 0.1 N NaOH and boiled for 5 min. The total solution was then cooled at room temperature and centrifuged for 15 min at 1000× g. The supernatant was collected and total protein content of each sample was measured according to the standard method (Lowry et al.1951).

*2.2.1.3. Determination of carbohydrate*

The carbohydrate content of every sample was determined by the following equation (Raghuramulu et al. 2003).

$$\text{Carbohydrate (g/100 g sample)} = \frac{100-(\text{moisture} + \text{fat} + \text{protein} + \text{ash} + \text{crude fibre})\ \text{g}}{100\ \text{g}}$$

*2.2.1.4. Determination of total lipid*

About 5 g of sample was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture, mixed thoroughly and let stand for 3 days. Then, the mixed solution was filtered and the filtrate was centrifuged for 15 min at 1000× g. After that the upper layer of methanol was removed by a Pasteur pipette and the chloroform remained at bottom portion was then evaporated by heating. The final pellet was the crude lipid. Next the lipid content was determined by the method of Folch et al. (1957).

*2.2.1.5. Determination of crude fibre and total ash*

The estimation of crude fibre and total ash content of the samples was done by the method as described by Raghuramulu et al. (2003).

10 gm of sample was taken in a beaker and 200 ml of boiling 0.255 N $H_2SO_4$ was added. The mixture was boiled for 30 minutes maintaining the volume constant by the addition of water at regular intervals. Then the mixture was filtered using a muslin cloth and the residue washed with hot water till the complete removal of acid. The washed residue was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added to it. After 30 minutes boiling (keeping the volume constant by adding water) the mixture was filtered using a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. After that the residue is transferred to a crucible, dried overnight at 80~100°C and weighed (We)in an electric balance. Then the crucible was heated at 400°C for 5~6 hours, cooled and weighed again (Wa). The amount of crude fibre was determined by the following formula.

Crude fibre (g/100 g sample) = [100 − (moisture + fat)] × (We-Wa)/Wt of sample

For the determination of ash content, 1 gram of the sample was weighed accurately into a crucible. The crucible was placed on a clay frame and first heated over a low flame till all the material was completely charred, followed by heating for 5~6 hours at 400°C. It was then cooled and weighed. To ensure completion of ashing, the crucible was then heated for 1 h, cooled and weighed. This was repeated till two successive weights were the same and the ash was more or less white in colour. Then total ash content was calculated as:

Ash content (g/100 g sample) = weight of ash × 100/weight of sample taken

### *2.2.2. Analysis of major phenolic compounds*

#### *2.2.2.1. Preparation of extract*

The extract was prepared following the method of Giri et al. (2012). Dried and powdered fruit bodies of *T. heimii* and *V. volvacea* were extracted with methanol at 25 °C for 24 hrs and then filtered with Whatman No. 1 filter paper. The full procedure was repeated twice. After filtration the filtrate was concentrated by a rotary evaporator under vacuum and stored at - 20 °C until further use.

#### *2.2.2.2. Total phenol determination*

Contents of total phenols were estimated according Folin-Ciocalteu method (Singleton et al. 1999). 1 ml of methanolic extract from each mushroom was mixed with 1 ml of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20 % $Na_2CO_3$ solution was added to the mixture and adjusted the final volume to 10 ml with distilled water. The mixture was allowed to stand at room temperature for 30 min in dark condition. Absorbance was measured against the blank reagent at 725 nm in spectrophotometer (TECHCOMP, Japan). Calibration curve is made using Gallic acid with a concentration range of 50- 1000 µg/ml. Results were expressed in mg gallic acid equivalent (GAE)/100g DW (Dry Weight).

*2.2.2.3. Total flavonoids determination*

Total flavonoids content was estimated according to the method used by Meda et al. (2005). 0.5 ml of methanolic extract of each mushroom was diluted by adding 0.5 ml of distilled water. Then, 0.5 ml of 10 % aluminium chloride (P/V) and the same volume of 1M sodium acetate were added. Finally, 2 ml of distilled water was added and after 30 min the absorbance was measured at 415 nm against a blank sample consisting of 4 ml methanolic extract. Quercetin with a concentration range of 0-100 µg/ml was used to draw the calibration curve. Results were expressed in mg of quercetin equivalent (QE)/100g DW.

*2.2.2.4. Determination of ascorbic acid content*

The quantity of ascorbic acid was determined following the method of Rekha et al. (2012). Standard used for this analysis contained ascorbic acid (100 µg/ml) made up to 10 ml with 0.6% oxalic acid. It was titrated with 2, 6- dichlorophenol indophenol dye. The amount of dye consumed ($V_1$ ml) is equivalent to the amount of ascorbic acid. Similarly, sample (W µg/ml) was titrated with dye ($V_2$ ml). The amount of ascorbic acid was then calculated by the formula,

Ascorbic acid (µg/mg) = [{(10 µg/$V_1$ml) × $V_2$ml} × W µg] × 1000

***2.2.3. Antioxidant assay***

The antioxidant activity of *T. heimii* and *V. volvacea* were measured by FRAP and DPPH assay.

*2.2.3.1. Ferric ion reducing antioxidant power (FRAP) analysis*

The FRAP assay measures the reduction of a ferric salt to the ferrous complex (blue coloured) by antioxidants under acidic condition (pH 3.6). The FRAP unit is defined as the reduction of one mole of $Fe^{3+}$ to $Fe^{2+}$. Ferric reducing ability determines the reducing capacity of an antioxidant. 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ (2,4,6-Tripyridyl-S-

Triazine) solution and 1 part of 20 mM $FeCl_3$, $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ solution is used for calibration. The antioxidant capacity based on the capacity to reduce ferric ions of sample is measured from the linear calibration curve and expressed in mM $FeSO_4$ equivalents per gram of sample. Ascorbic acid used as a positive control.

*2.2.3.2. DPPH (Di picryl phenyl hydrazine) radical scavenging activity*

This method uses stable chrome radical, DPPH in methanol, which give deep purple colour, when the DPPH solution is mixed with a substance that can donate a hydrogen atom, it becomes reduced accompanied by loss of deep purple colour. This decrease in absorbance is characterized by an absorption band at about 515 nm. The reaction mixture (3.0 ml) consists of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of the extract and 1.0 ml of methanol. The whole mixture was incubated for 10 min in dark, and then the absorbance is measured at 515 nm. In this assay, the positive control was ascorbic acid (Blois 1958). The percentage of inhibition can be calculated using the formula:

Inhibition % = $\{(A_0 - A_1)/ A_0\} \times 100$

Where, $A_0$ is the absorbance of control and $A_1$ is the absorbance of test. This assay is simple and widely used. The $IC_{50}$ value was also calculated for both samples.

***2.2.4. Fatty acid analysis***

*2.2.4.1. Preparation of FAMEs*

Dried mushroom powders of *T. heimii* and *V. volvacea* were used to prepare fatty acid methyl esters (FAMEs) by direct (*in-situ*) trans-esterification (Khot et al. 2017). In brief, reaction mixture containing methanol, chloroform and 0.2 M methanolic sulfuric acid (10:1:1, v/v/v) was added per g of sample. The suspension was sonicated at 50 kHz and 30°Cfor 10 min after which it was refluxed for 20 hrs at 65°C under atmospheric pressure and stirred conditions. The resulting mixture was filtered and the residue derived from

vacuum evaporation was dissolved in chloroform: methanol (2:1, v/v). The water-soluble impurities, if any, were removed from the sample by washes with purified water until neutrality was obtained. The organic phase was separated, dried over anhydrous sodium sulphate and FAME was obtained after rotary evaporation (EYELA, Japan) for further analysis.

*2.2.4.2. Fatty Acid Profiling*

FAME derived from dried mushroom powder were analysed for fatty acid composition using gas chromatograph (Scion 436-GC, Scion Instruments, Netherlands) with a capillary column, Rt-2560 (100m×0.25mmID×0.2 μm; Restek Corp., USA); flame ionization detector (FID) and helium as the carrier gas (injector temperature: 225 °C; detector temperature: 285 °C). The column temperature was initially kept at100 °C and then upgraded to 240 °C at a rate of 3 °C/min and maintained for 15 min. The fatty acid identification was carried out by comparing retention times of resolved components with that of an authentic standard (Supelco® 37 Component FAME Mix, 47885-U, Sigma–Aldrich), a 37-component mixture of saturated, mono- and polyunsaturated fatty acid methyl esters.

### *2.2.5. EDAX analysis*

The Energy Dispersive X-ray (EDAX) microanalysis is a technique of elemental analysis based on the generation of characteristic X-rays that reveals the presence of specific elements within the test samples (Turhan et al.2010).In the present study EDAX analysis (FEI, Netherlands) was performed to determine the major elements present in *T. heimii* and *V. volvacea.*

## 2.3. Results and Discussions

### *2.3.1. Nutrient content*

The proximate composition and nutrient contents of the 9 studied mushroom species has displayed in Table 2.1. Energetic contributions of the mushrooms differ from species to species due to their nutritional contents. In the present study the protein content ranged 20.4-39.2% on dry weight basis. In particular, the species like *T. heimii* and *V. volvacea* possess high amount of protein content (39.2% and 37.5% repectively). The lipid content in the studied mushrooms showed very low amount ranging 0.8-3.4%. The results have revealed that the carbohydrate content is relatively higher in *T. heimii* and *V. volvacea* than other species and recorded *as* 42.6% and 43.4% respectively (Table 2.1).

Generally, mushrooms contain 19% - 35% protein, as compared to 7.3% in rice, 13.2% in wheat, 25.2% in milk, 39.1% in soyabean and 62% in meat on dry weight basis (Chang and Miles 1992). Samajipati (1978) has reported 30.16%, 28.16%, 34.7% and 29.16% protein in dried mycelium of *A. campestris, A. arvensis, M. esculenta* and *M. deliciosa* respectively. Therefore, in terms of protein content, mushrooms rank below animal meats but well above most other foods materials including milk. Protein content in *Pleurotus* sp. has been documented to range between 8.9 and 38.7% on dry weight basis (Bano and Rajarathnam 1982). In *Agaricus bisporus, Lentinus subnudus, Calocybe indica* and *Volvariella volvacea* the crude protein ranged 14% - 27% on dry weight basis (Purkayastha and Chandra 1985). Rai and Sohi (1988) also reported protein content of *Agaricus bisporus* to be 29.3% on dry weight basis. Sharma et al. (1988) has estimated 14.71%- 17.37% and 15.20%- 18.87% protein in the fruiting bodies of *Lactarious deliciosus* and *Lactarious sanguiffus* respectively. Nutritional analysis of two wild edible mushrooms (*Schizophyllum commune* and *Lentinula edodes*) from northeast region of India have been studied by Longvah and Deosthale (1998) and reported that protein content of *L. edodes* (26%) is much higher than the *S. commune* (16%). Nutritional values of seven edible wild mushrooms were analysed by Agrahar-murugkar and Subbulakshmi (2005) which are commonly consumed in

the Khasi hills of Meghalaya and reported that 27.3, 27.5, 21.1, 24.1, 21.1, 21.2, 19.0% protein present in *Calvatia gigantea, Clavulina cinerea, C. cibarius, Ramaria brevispora, Russula integra, Gomphus floccosus* and *Lactarius quieticolor* respectively. Earlier Pushpa and Purushothama (2010) have analysed the nutritional composition of five mushroom species and found 21.60, 41.06, 27.83, 26.25, 18.31% protein in *C. indica, A. bisporus, Pleurotus florida, Russula delica* and *Lyophyllum decastes* respectively.

The carbohydrate content of mushrooms constitutes the bulk of fruiting bodies having more than 50% on dry weight basis. Nutritional analysis of two edible wild mushrooms (*Schizophyllum commune* and *Lentinus edodes*) from northeast region of India have been analysed by Longvah and Deosthale (1998) and reported that total carbohydrate content in *L. edodes* and *S. commune* were 64.4% and 68% respectively. Jagadeesh et al. (2010) reported that 34.75 and 38.9% of carbohydrate content present in mycelia and fruit body of *Volvariella bombycina*. Pushpa and Purushothama (2010) have analysed the nutritional composition of five mushroom species and found 49.20, 28.38, 32.08, 34.88, 34.36% carbohydrate content in *C. indica, A. bisporus, P. florida, R. delica,* and *L. decastes* respectively. Manjunathan and Kaviyarasan (2011) have determined the nutrient composition of *L. tuberregium* in both wild and cultivated type and found 58.05 and 55.8% carbohydrate in cultivated variety and in wild variety respectively. Nutritional values of wild mushrooms have been studied by Johnsy et al. (2011) and found good source of carbohydrates ranged from 33.23% within *A. auricula* to 50.2% within *L. tuber-regium.* Kumar et al (2013) has reported the carbohydrate contents of 15 selected mushrooms from Nagaland, India ranged from 32.43% in *S. commune* to 52.07% in *Boletus aestivalis*. In this study, carbohydrate content ranged from 33.2% to 43.4% in dry weight basis. The carbohydrate content reported in the samples is an evidence of their being highly nutritious and good for human consumption.

Generally, in mushrooms, the lipid content is very low as compared to proteins and carbohydrates. In the present investigation lipid content of the studied mushroom species were ranged from 0.8% to 3.4%, which is almost similar with previous studies done by different workers. Singer (1961) has determined the lipid content in *Suillus granulates* as 2.04%, *Suillus luteus* as 3.66% and *A.* campestris as 2.32%. Crude lipid content ranging from 1.08 to 9.4% with an average of 2.85% has been reported in *Pleurotus* species by Bano and Rajarathnam (1982). On fresh weight basis, the lipid content of 0.10 to 0.19% in *Pleurotus* species has been reported by Rai et al. (1988). Longvah and Deosthale (1998) have reported that crude lipid content (2%) were similar in two edible wild mushrooms (*S. commune* and *L. edodes*) from northeast India. Lipid content of fresh *A. bisporus* and *P. ostreatus* was analysed by Manzi et al. (2001) and found 0.3 and 0.4 g/100 g, respectively. Agrahar-murugkar and Subbulakshmi (2005) also reported the fat content (ranged from 1.0% in *C. gigantean* to 5.3% in *G. floccocus*) of seven different wild mushrooms collected from the Khasi hills of Meghalaya.

In the present study, selected mushroom species showed significant values for fibre and ash contents (varied from 2.0 - 8.6% and 2.3 - 11.5% respectively) on dry weight basis. These results indicated that the wild edible mushrooms of Gurguripal ecoforest are good source for crude fibres and minerals. Mushrooms contain a relatively high amount of fibre which may accounts for its relatively high amount of ash (Cheung 1998). The ash content recorded 16.48 and 14.93 g/100g in the wild edible mushrooms such as *A. silvaticus* and *A. silicola* respectively (Kalac 2009). These values agreed well with those reported by Bernaś and Jaworska (2010). The values of fibre and ash content were 13.21% and 7.97% for *Agaricus bisporus*, and 8.25% and 5.86% for *Pleurotus ostreatus*, respectively (Raya et al. 2014).

The studied mushroom species from Gurguripal ecoforest have high protein and carbohydrate contents in contrast to low fat levels, which make them suitable as highly nutritious and low-calorie diets. In the present study, *T. heimii* showed highest protein content above all 9 species, which is in agreement with the reports of Parent and Thoen (1977).

Moreover, it was observed that the total protein content of *T. heimii* (39.2%) is almost similar with that of soyabean (39.1%). These findings correlate the fact of high demand and price of *T. heimii* in commercial market. Since the nutrient contents were higher in *T. heimii* and *V. volvacea* in comparison with other species, have been selected for further bioactive component analysis.

**Table 2.1-Nutrient contents of prime edible mushrooms in Gurguripal**

| Mushroom | Protein (%) | Carbohydrate (%) | Lipid (%) | Fibre (%) | Ash (%) |
|---|---|---|---|---|---|
| ***Agaricus* sp.** | 33.8±2.861 | 36.2±1.233 | 3.4±0.411 | 8.6±2.351 | 5.7±2.327 |
| ***Amanita bisporigera*** | 20.4±3.594 | 34.5±2.001 | 0.8±0.159 | 6.7±1.302 | 10.4±1.147 |
| ***Astraeus hygrometricus*** | 27.8±2.368 | 37.3±0.865 | 2.06±0.857 | 3.4±1.143 | 5.8±1.47 |
| ***Cantharellus* sp.** | 34.1±0.981 | 38.8±1.349 | 1.4±0.613 | 2±1.247 | 8.2±1.225 |
| ***Termitomyces medius*** | 28.2±2.613 | 39.4±2.042 | 1.9±1.062 | 4.7±1.429 | 8.3±1.878 |
| ***Pleurotus ostreatus*** | 32.3±3.675 | 38.6±2.858 | 2.4±0.49 | 4.3±0.984 | 10.8±1.023 |
| ***Schizophyllum commune*** | 20.6±2.042 | 33.2±2.861 | 1.8±0.899 | 6.2±1.837 | 11.5±1.715 |
| ***Termitomyces heimii*** | 39.2±1.233 | 42.6±1.473 | 2.14±0.816 | 7.8±1.347 | 3.7±1.47 |
| ***Volvariella volvacea*** | 37.5±1.184 | 43.4±1.226 | 2.6±0.66 | 5.6±1.801 | 2.3±1.184 |

Data represents the mean values ± standard deviation (SD) of triplicate results. Significantly higher ($p < 0.01$) carbohydrate and protein contents were observed in *V. volvacea* and *T. heimii* respectively

### *2.3.2. Phenolic compounds*

Quantitative values of major phenolic compounds present in *T. heimii* and *V. volvacea* were shown in Table 2.2. The methanolic extracts of these mushrooms were rich in phenols followed by flavonoids and ascorbic acid. Phenolic compounds are considered as powerful chain-breaking antioxidants and their hydroxyl groups provide scavenging ability.

The studied wild mushrooms are vital source of bioactive compounds that were quantified in terms of total phenol and flavonoids with their bioactive properties such as antioxidant and antibacterial potential and the results are in agreement with other reports concerning to bioactive compounds (phenols and flavonoids) quantification in different mushrooms (Caglarlrmark et al. 2002; Barros et al. 2007a; Barros et al. 2008). In the present study, methanolic extract from the whole fruit body of mushroom was extracted, considering that the mixture of phytochemicals may have potential additive or synergistic effects. The bioactivity of phenolic compounds may be related to their metal chelating and free radical scavenging ability (Decker 1997). Flavonoids can function as free radical scavengers by terminating the radical chain reactions occur during the oxidation of triglycerides in our body (Roedig-Penman and Gordon 1998). These molecules can also play protective role in diseases related to oxidative stress, such as cancer and cardiovascular diseases (Ferreira et al. 2009; 2010).

Several references are available on the positive correlation between total content of phenolics in the mushroom extracts and their antioxidant activity. For instance, Puttaraju et al. (2006) studied 23 mushroom species; among them *Termitomyces heimii* and *Helvella crispa* ranked as high-phenolics species and accordingly, by all assays *T. heimii* and *T. mummiformis* were exhibited higher antioxidant activity.

Cheung et al. (2003), reported total phenolic contents in *Lentinus edodes* and *V. volvacea* extracted with different solvents were higher in methanolic solvent extracts (4.79-15.0 mg/g) than in water extracts (1.33-1.34 mg/g), where total phenolic content was three times higher in *V. volvacea* than in *L. edodes*. Kalava and Menon (2012) reported, total phenols and flavonoid content in the aqueous extract of the mycelium of *V. volvacea* were 19.08 and 8.23 mg/g, respectively. Later, Punitha and Rajasekaran (2014) measured the amount of total phenolics, flavonoids and ascorbic acid of hot water and methanolic extraction of *V. volvacea*. In case of methanolic extract, phenolic content is 53.13 mg/g, flavonoid content is 14.35 mg/g, and ascorbic acid is 1.72 mg/g.

Earlier, Mitra et al. (2015) reported total phenols 2049 ± 99µg/gm, total flavonoids 840 ± 15µg/gm and ascorbic acid 105.9 ± 14.7µg/gm from hot water extract of *T.heimii*. Tripathy et al. (2016) has also measured the phenolic compounds from methanolic extract of *T. heimii* as total phenols 2440 ± 10.0µg/gm, total flavonoids 550 ± 16.0 µg/gm and ascorbic acid 75 ± 4.0 µg/gm. These variations of phenolic contents in *T. heimii* are probably due to different soil compositions of their habitat. Phenols and flavonoids have anti-carcinogenic and anti-mutagenic effects by protecting DNA against free radicals. In particular flavonoids and ascorbic acid have a synergistic protective effect towards oxidative damages of DNA in lymphocytes (Noroozi et al. 1998; Lairon and Amiot 1999). It has been established that free radical-scavenging activity is immensely influenced by the phenolic composition of samples (Cheung et al. 2003) and the reducing power exhibited by mushrooms is due to their hydrogen-donating ability (Shimada et al. 1992). In this regard *T. heimii* and *V. volvacea* are rich in phenolic compounds, thus can be potential natural resource with immense health protective attributes.

**Table 2.2 - Extraction yield of phenolic compounds from *T. heimii* and *V. volvacea* (µg/gm)**

| Mushroom | Total Phenols | Total flavonoids | Ascorbic Acid |
|---|---|---|---|
| ***T. heimii*** | 2365±12.34 | 1050.33±16.73 | 97.94±3.12 |
| ***V. volvacea*** | 1389±8.98 | 987±12.23 | 42.70±4.65 |

### *2.3.3. Antioxidant assay*

The DPPH radical is a stable free radical having an odd electron and shows distinct absorption band at 517 nm. Methanolic DPPH is violet in colour and ultimately becomes yellowish when DPPH acts as an electron acceptor. In present study, methanolic extract of *T. heimii* and *V. Volvacea* act as electron donor and finally stabilize the DPPH molecules. Here, *T. heimii* showed the lower value ($IC_{50}$= 43.32±3.47 mg/gm) than *V. volvacea* ($IC_{50}$= 48.58±3.21 mg/gm). In case of FRAP analysis, the result also showed lower value of *T. heimii* ($IC_{50}$= 51.39±2.62 mg/gm) than *V. volvacea* ($IC_{50}$= 55.64±3.19 mg/gm).

The antioxidant potential from water and methanolic extracts of fruiting bodies of 23 species of mushrooms was measured by Puttaraju et al. (2006), out of them *T. heimii* showed moderately lower $IC_{50}$ value both in water and methanolic extract than the others. The $IC_{50}$ value of methanolic extract of *V. volvacea* was 110.40 mg/ml and hot water $IC_{50}$ concentration for DPPH was 142.45 mg/ml (Punitha and Rajasekaran 2014). DPPH radical scavenging ability of polyphenolic rich extract of *T. heimii* showed the $EC_{50}$ value of 0.49 ± 0.02 mg/ml (Mitra et al. 2015). Ethanol and aqueous extract of *T. clypeatus* exhibited $IC_{50}$ value of 0.86 and 0.27 mg/ml in scavenging DPPH radicals (Mondal et al. 2016). Previously

Mathew et al. (2015) evaluated the antioxidant activity of ethanolic extract from *V. volvacea* through various in vitro hydroxyl radical scavenging, DPPH and FRAP assays. Earlier Tripathy et al. (2016) reported slightly higher value of FRAP by *T. heimii* ($IC_{50}$ value= 0.59 ± 0.04 mg/ml). A much higher value of FRAP ($IC_{50}$ value = 1.77 ± 0.035 mg/ml) was shown by ethanolic extract of *T. clypeatus* (Mitra et al. 2016).

Lower values of DPPH and FRAP assay indicate higher radical scavenging ability. In this regard the methanol extract of *T. heimii* showed better scavenging activity both in terms of DPPH scavenging and FRAP assay than *V. volvacea*. Free radical scavenging activity has been recognized as an established phenomenon in inhibiting lipid oxidation, otherwise which can be detrimental to the cellular components and cell functioning (Puttaraju et al. 2006). The extracts of two over mentioned mushroom species showed effective antioxidant capacity which may be contributed by their rich phenolic contents (Ferreira et al. 2009). Earlier, Pietta (2000) has reported that the phenolic compounds in plants were accountable for most of the antioxidant activities whereas Mau et al. (2001) showed that the effective bioactive substance in mushrooms are mainly phenolic compounds with strong antioxidant activity. The hydroxyl groups on benzene ring of phenolic acids has the ability to chelate metals involved in production of free radicals, therefore, are responsible for antioxidant properties (Pereira et al. 2012; Khatua et al. 2013). Altogether these studies might explain the fact that higher antioxidant property is due to higher content of phenolics in mushrooms, confirms the reason of higher antioxidant activity of *T. heimii* as it possess higher phenolic content than *V. volvacea* (Table 2.2).Thus in particular the consumption of *T. heimii* might be beneficial to protect human body against oxidative damage and avoid health related degenerative illness.

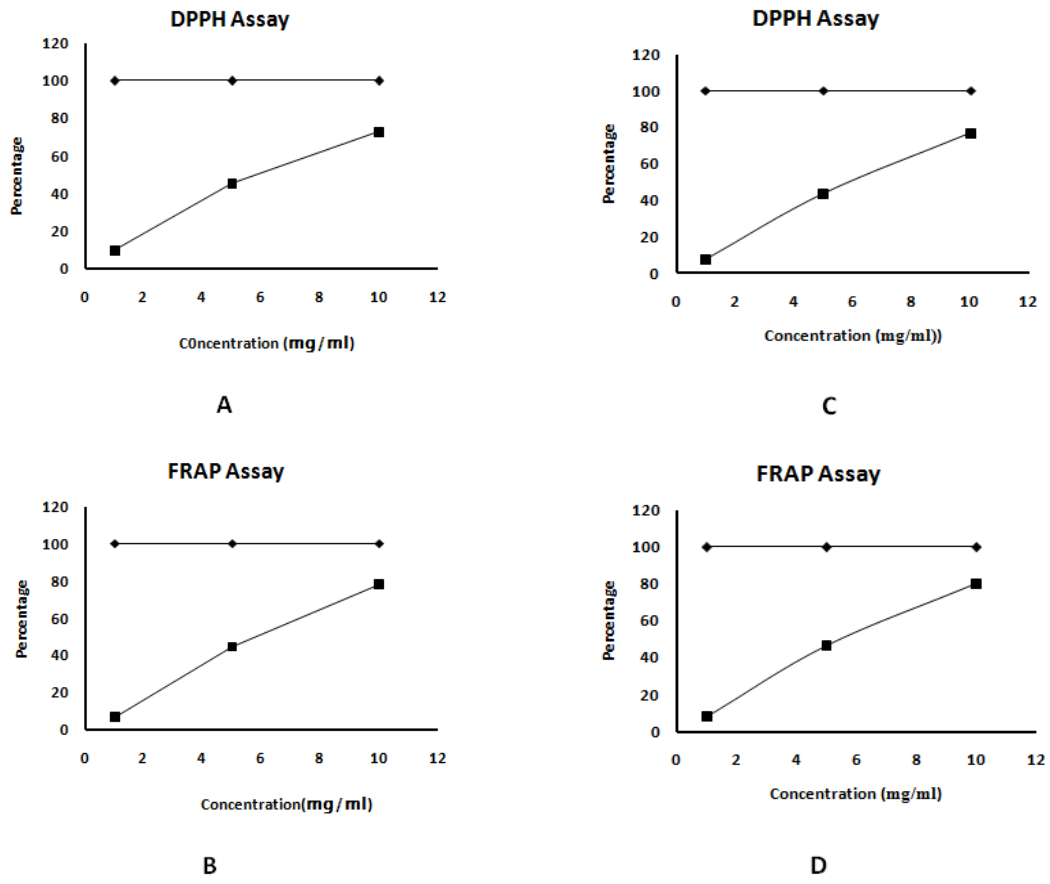



**Fig 2.1- Antioxidant assay ($IC_{50}$ value in %) of methanolic fractions of *T. heimii* (A, B) and *V. volvacea* (C, D)**

### *2.3.4. Fatty acids*

The FAME of the hot water extract from *T. heimii* and *V. volvacea* was analysed through gas chromatography and a total of 11 peaks were noted in each, among them two SFA (Palmitic acid and Stearic acid), two MUFA (Oleic acid and Palmitoleic acid) and one PUFA (Linoleic acid) identified by authentic standards. The results revealed that higher quantity of unsaturated fatty acids (MUFA and PUFA) is present in *T. heimii*. The most abundant fatty acid in *T. heimii* was recorded as linoleic acid (33.53µg/ml) followed by oleic acid (25.08µg/ml) and palmitic acid (15.60 µg/ml). Johnsy and Kaviyarasan (2015) measured fatty acids of two different species of the genus *Termitomyces* (*T. microcarpus* and *T. heimii*) and found that the amount of the linoleic acid (20.54 µg/ml) in *T. heimii* was highest,

followed by palmitic acid (11.29 µg/ml) and oleic acid (11.13 µg/ml). The variation in fatty acid composition within the same species probably due to the variation of soil composition in their natural habitat.

In case of *V. volvacea* the most abundant fatty acid is stearic acid (87.65µg/ml) followed by oleic acid (16.55µg/ml), linoleic acid (9.67 µg/ml) and palmitic acid (3.43µg/ml). The present findings revealed that *V. volvacea* is rich in saturated fatty acid (SFA) rather than unsaturated fatty acids (UFA). These results are in strong agreement with the earlier reports that many mushroom species have high proportions of unsaturated fatty acids, particularly the linoleic acid (Kalaˇc 2009, Ruess et al. 2002). Linoleic acid is considered as the principal aromatic compound in most fungi and might contribute to the unique flavour of mushrooms (Maga 1981). Linoleic acid is also important for growth and maintenance of health through the synthesis of prostaglandins; regulation of blood pressure, immune response, inflammation, and apoptosis. Further, the consumption of *T. heimii* would be beneficial for health purposes and may have chemo preventive properties of selected diseases of humankind (Malek et al. 2012). Oleic acid is a bioactive compound, strongly inhibits the activity of human telomerase in a cell free enzymatic assay (Oda et al. 2002) and also may hinder the progression of adrenoleukodystrophy, a fatal disease that detrimental to brain and adrenal glands (Dogˇan and Akbaş 2013). More amount of oleic acid is present in *T. heimii* (25.08 µg/ml) when compared to the *V. volvacea* (16.55 µg/ml).

The present study has indicated that economically important and edible mushrooms demonstrate significant antioxidant features as well as could be a good source of health promoting unsaturated fatty acids. Finally, as per the results, due to presence of higher quantity of unsaturated fatty acids (MUFA and PUFA) *T. heimii* is preferred over *V. volvacea* as healthy cholesterol free diet.

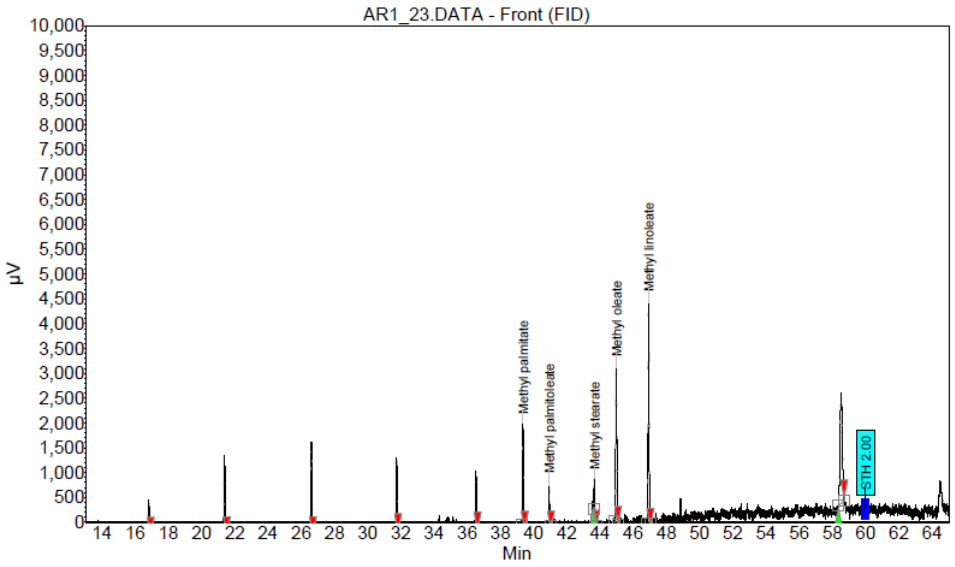



**Fig 2.2(A): Chromatogram of GC-MS analysis of methanolic extracts of *T. heimii***

**Table 2.3 (A)- Analysis of fatty acids in *T. heimii* through GC-MS**

| Index | Name | Time [Min] | Quantity [ug/ml] | Height [µV] | Area [µV.Min] | Area % [%] |
|---|---|---|---|---|---|---|
| 1 | UNKNOWN | 16.83 | 0.00 | 655.2 | 46.2 | 2.923 |
| 2 | UNKNOWN | 21.39 | 0.00 | 1541.8 | 117.5 | 7.441 |
| 3 | UNKNOWN | 26.61 | 0.00 | 1827.7 | 126.3 | 7.992 |
| 4 | UNKNOWN | 31.75 | 0.00 | 1392.0 | 92.6 | 5.863 |
| 5 | UNKNOWN | 36.52 | 0.00 | 1148.8 | 74.1 | 4.688 |
| 6 | Methyl palmitate | 39.36 | 15.60 | 2097.1 | 141.6 | 8.967 |
| 7 | Methyl palmitoleate | 40.94 | 5.85 | 848.8 | 50.2 | 3.176 |
| 8 | Methyl stearate | 43.66 | 4.63 | 732.3 | 41.8 | 2.644 |
| 9 | Methyl oleate | 44.98 | 25.08 | 3126.7 | 229.9 | 14.554 |
| 10 | Methyl linoleate | 46.93 | 33.53 | 4439.4 | 299.2 | 18.944 |
| 11 | UNKNOWN | 58.53 | 0.00 | 2204.2 | 360.3 | 22.808 |
| | | | | | | |
| Total | | | 84.69 | 20014.0 | 1579.6 | 100.000 |

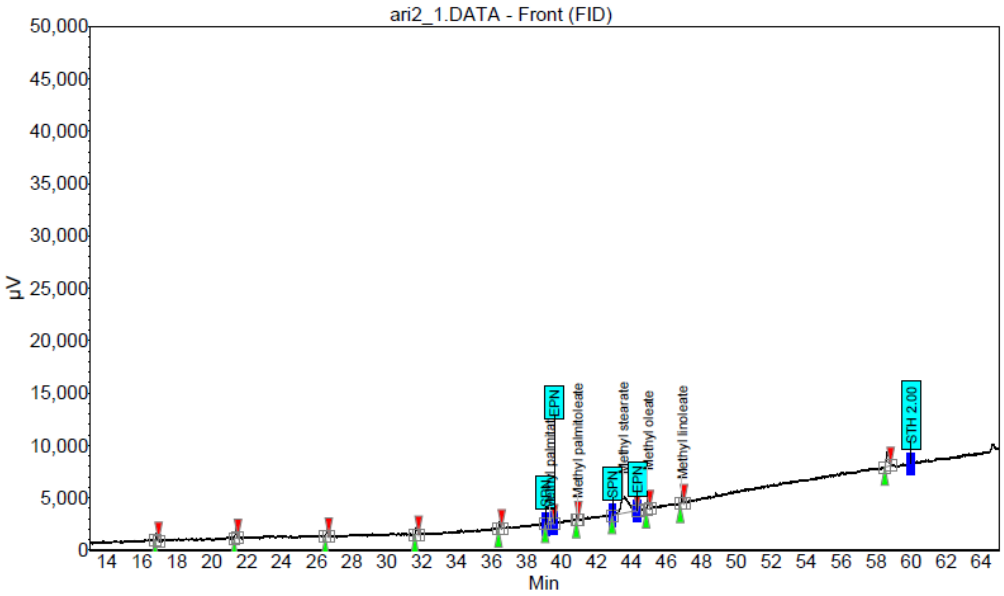


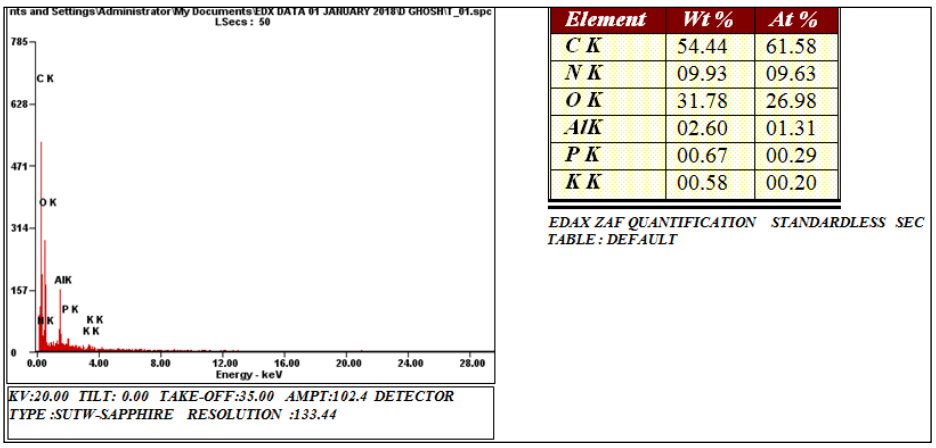

**Fig 2.2(B): Chromatogram of GC-MS analysis of methanolic extracts of *V. volvacea***

**Table 2.3 (B) - Analysis of fatty acids in *V. volvacea* through GC-MS**

| Index | Name | Time [Min] | Quantity [ug/ml] | Height [µV] | Area [µV.Min] | Area % [%] |
|---|---|---|---|---|---|---|
| 1 | UNKNOWN | 16.84 | 0.00 | 702.8 | 50.4 | 2.926 |
| 2 | UNKNOWN | 21.38 | 0.00 | 1443.7 | 104.3 | 6.056 |
| 3 | UNKNOWN | 26.60 | 0.00 | 1495.6 | 105.8 | 6.146 |
| 4 | UNKNOWN | 31.72 | 0.00 | 1289.5 | 82.8 | 4.809 |
| 5 | UNKNOWN | 36.49 | 0.00 | 947.7 | 57.2 | 3.320 |
| 6 | Methyl palmitate | 39.31 | 3.43 | 320.7 | 31.1 | 1.806 |
| 7 | Methyl palmitoleate | 40.89 | 3.94 | 632.1 | 33.8 | 1.961 |
| 8 | Methyl stearate | 43.51 | 87.65 | 1563.7 | 791.2 | 45.948 |
| 9 | Methyl oleate | 44.93 | 16.55 | 1845.3 | 151.7 | 8.810 |
| 10 | Methyl linoleate | 46.89 | 9.67 | 1236.0 | 86.3 | 5.013 |
| 11 | UNKNOWN | 58.65 | 0.00 | 1409.7 | 227.4 | 13.205 |
| | | | | | | |
| Total | | | 121.23 | 12886.8 | 1721.9 | 100.000 |

### *2.3.5. Elemental constituents*

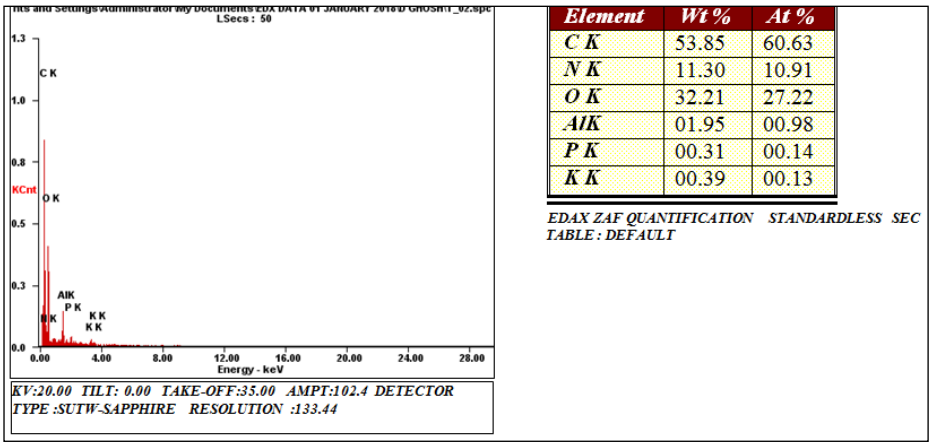
The mean elemental concentrations with their standard deviations dry mass of *T. heimii* and *V. volvacea* were represented in Table 2.4 (A) and 2.4 (B) respectively. The concentrations of C, N, O, Al, P, K were determined. The micronutrient analysis of two mushrooms showed that phosphorus (P) and potassium (K) content of *T. heimii* is significantly higher than *V. volvacea*. These two essential microelements are very important to maintain the water and acid-base balance in blood and tissues. In both the mushroom the carbon, oxygen and nitrogen content present in very high percentage. Among them carbon content is highest followed by oxygen and nitrogen sequentially.




| Element | Wt % | At % |
|---|---|---|
| C K | 54.44 | 61.58 |
| N K | 09.93 | 09.63 |
| O K | 31.78 | 26.98 |
| AlK | 02.60 | 01.31 |
| P K | 00.67 | 00.29 |
| K K | 00.58 | 00.20 |

EDAX ZAF QUANTIFICATION STANDARDLESS SEC TABLE : DEFAULT

**Fig 2.3(A) Nutrient analysis of *T. heimii* through EDAX**




| Element | Wt % | At % |
|---|---|---|
| C K | 53.85 | 60.63 |
| N K | 11.30 | 10.91 |
| O K | 32.21 | 27.22 |
| AlK | 01.95 | 00.98 |
| P K | 00.31 | 00.14 |
| K K | 00.39 | 00.13 |

EDAX ZAF QUANTIFICATION STANDARDLESS SEC TABLE : DEFAULT

**Fig 2.3(B) Nutrient analysis of *V. volvacea* through EDAX**

## 2.4. Conclusion

The use of mushrooms as food is probably as old as civilization and currently gained greater importance in the diet of mankind. The prime edible mushrooms of Gurguripal ecoforest showed promising nutritional attributes and consumed as substitutes of animal protein. Among them two edible species *T. heimii* and *V. volvacea* were the most favoured species on the basis of taste and proximate composition. Those two species were also rich in phenolic compounds which impart their antioxidant capability. Based on the fact that free radicals are highly dangerous to normal human physiology, high phenolic content of *T. heimii* and *V. volvacea* have enormous health benefits to us. Furthermore, the abundant presence of linoleic acid (PUFA) in *T. heimii* established its immense value as low cholesterol dietary supplement. Altogether, the overall findings of the present chapter suggested that due to presence of higher nutritional attributes as well as remarkable antioxidant properties ***Termitomyces heimii*** is preferred over all the studied mushroom species for health promotion.