Evaluation of Antioxidant Activities and Determination of Bioactive Compounds in Two Wild Edible Termitomycetes (*T. microcarpus* and *T. heimii*)

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Abstract: Mushrooms have a long traditional use in many countries. They are food full of proteins, rich in vitamin B, rich in different minerals and have almost all essential amino acids. Mushrooms have been reported as useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. To investigate the effect of two wild edible *Termitomycetes* species were collected from different site/forest areas of Western-ghats of Kanyakumari district and then were evaluated for antioxidant activity. The two *Termitomycetes* species viz., *T. microcarpus* and *T. heimii* were collected in wild and dried in an oven at 40°C. The dried powdered samples were extracted with methanol. The extracts were used for different antioxidant assays such as, ABTS+ Radical scavenging activity, DPPH radical scavenging activity, reducing power assay, chelating effects on ferrous ions, superoxide radical scavenging activity, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity and determination of antioxidant compound using standard methods. It was revealed from the study that all the concentrations of tested mushroom extracts showed antioxidant activity. However, the highest antioxidant activity was observed (200 µg/mL) in mushroom extracts. Total phenols were the major antioxidant compound detected in methanolic extracts. In the present study, the test fungus *T. microcarpus* and *T. heimii* seemed to store more antioxidant compounds (phenol 10.47 and 12.26, flavonoids 2.14 and 2.81, β-carotene 0.2 and 0.25, lycopene 0.37 and 0.25, ascorbic acid 1.06 and 1.31 mg/g). The findings show that the test organism showed the strong antioxidant activity. These mushrooms could be exploited for pharmacological and nutraceutical advantages.

Key words: *Termitomycetes* · Antioxidants · Free radicals · β-carotene · lycopene · Ascorbic acid

INTRODUCTION

The edible wild mushrooms command higher prices than cultivated mushrooms, people prefer to consume them due to their flavour and texture. Nevertheless, wild edible mushrooms are becoming more and more important in our diet for their nutritional and pharmacological characteristics [1, 2]. Antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage [3]. These free radicals are capable of damaging all components of body viz, lipids, protein, DNA and sugars [4]. These biomolecules contribute to prevention of coronary and vascular diseases and of tumor formation by inhibiting oxidative reactions [5].

Today it is known that a wide variety of pathological damage, such as carcinogenesis and rheumatoid arthritis, can be caused by oxygen derived free radicals. In living systems some free radical species, for example OH radical can cause lipid peroxidation that is oxidative modification of low-density lipoproteins (LDLs). This process may play role in the development of arthrosclerosis. From all the free radical species OH and O^{2-} radicals are found to be the main culprits in the damage that free radicals induced in biological systems. Almost all organisms are well protected against free radical damage by antioxidants systems such as enzymes, superoxide dismutase and catalase and/or by compounds such are ascorbic acid, tocopherols and glutathione. In spite of that sometimes mechanism of antioxidant protection becomes unbalanced

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and then antioxidants taken in by food playing important role in reducing oxidative damage-phenolic compounds, protein hydrolyzates and some amino acids, present in different food were found to have antioxidant properties.

The Western-ghats of Kanyakumari district provides a rich habitat for mushroom growth. Mushrooms which grow wild in Kanyakumari district have not been explored fully for antioxidant activity and other medicinal properties. Today, in order to find dietary sources full of antioxidants that can help human body to reduce oxidative damage. Therefore, an attempt was made to carry out the antioxidant activity of two wild edible *Termitomycetes* mushrooms were collected from forest areas of Western-ghats.

MATERIALS AND METHODS

Chemicals: Potassium ferricyanide, ferrous chloride, ferric chloride, Folin-Ciocalteu's reagent (FCR), methanol and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt. Germany). 1.1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH. Sternheim. Germany). All other chemicals and solvents are of analytical grade.

Mushrooms: Fruiting bodies of edible mushrooms viz. *T. microcarpus* and *T. heimii* were collected from forest area of Kanyakumari District in 2010 and 2011 and were authenticated based on their microscopic and macroscopic characteristics. They were stored at the laboratory herbarium of University of Madras. The fruiting body of mushroom samples were divided into parts and then air dried in an oven for 48 h at 40°C before analysis.

Extraction: Dried mushroom samples were grounded in a blender before the extraction process. Mushroom sample (10 g) was extracted by methanol (50 mL). After filtration a volume of obtained liquid extract was measured and extraction solvent was removed by rotary evaporator under vacuum. After that samples were dried at 60°C to the constant mass. In this way extraction yield was obtained. Dry extracts were placed into a glass bottles and stored at 4°C to prevent oxidative damage until analysis.

$ABTS^+$ Radical Cation Decolourisation: The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS)) assay was done as previously described [6]. Briefly, the stock solutions were 7.4 m mol/L $ABTS^+$ and 2.6 m mol/L potassium per sulfate. The working solution was then prepared by mixing the two stock solutions in equal volumes and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^+$ solution with 60 mL methanol to obtain an absorbance of 1.1 at 734 nm. A fresh $ABTS^+$ solution was prepared for each assay. A volume of 150 mL of each extract (final concentrations from 5 to 100 mg/mL) was allowed to react with 2850 mL of the $ABTS^+$ solution (final concentration of 0.02mol/L) for 2 h in the dark. Finally, the absorbance at 734 nm was measured. Distilled water instead of mushroom extracts served as the control. The capability of the extracts to scavenge the ABTS radical was calculated by applying the following equation:

$$ABTS^+ \text{ scavenging effect \%} = [(A_{Control} \_ A_{Sample} / A_{control}) \times 100]$$

Where, $A_{control}$ was the absorbance of the reaction in the presence of water and A_{sample} the absorbance of the reaction in the presence of the extract. The extract concentration producing 50% inhibition (EC_{50}) was calculated from the standard graph of the ABTS scavenging effect against the extract concentration. BHT was used as the positive control.

DPPH radical Scavenging Activity: The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay was done as described previously [6]. Briefly, the stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. A volume of 150 mL of each extract (final concentrations ranging from 50 to 800 mg/mL) was allowed to react with 2850 mL of the DPPH solution (final concentration of 0.1 m mol/L), vigorously shaken and maintained for 1 h at room temperature in the dark. Distilled water was used instead of extract as a control. Then the absorbance was measured at 515 nm. The capability of the extracts to scavenge the DPPH radical was calculated using the following equation:

$$DPPH \text{ scavenging activity(\%)} = [(A_{Control} \_ A_{Sample} / A_{Control}) \times 100]$$

Where, $A_{control}$ was the absorbance of the reaction in the presence of water and A_{sample} the absorbance of the reaction in the presence of the extract. The extract concentration producing 50% inhibition (EC_{50}) was calculated from the graph of the DPPH scavenging effect against the extract concentration. BHA was used as standards.

Reducing Power: The reducing power was assayed as described in Kuda *et al*. [7] with some modification. Different concentrations of ethanolic extracts of mushrooms (1.0 ml) were mixed with 2.5 mL of phosphate buffer (50 mM, pH 7.0) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. After the addition of 2.5 mL of trichloroacetic acid (10%), the mixture was then centrifuged at 200×g for 10 min. Finally, 1.25 mL supernatant was mixed with 1.25 mL of distilled water and 0.25 mL $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard.

Ferrous-ion Chelating Activity: The ferrous-ions chelating ability of extracts was determined as described by Soras *et al*. [6]. Briefly, a sample (0.7 mL) of each extract was diluted in 0.7 mL of distilled water and was mixed with 0.175 mL of $FeCl_2$ (0.5 m mol/L) and the absorbance (A_0) was read at 550 nm. After the reaction was initiated by the addition of 0.175 mL ferrozine (0.5 m mol/L), the mixture was shaken vigorously for 1 min and let stand at the room temperature for 20 min when the absorbance (A_1) was again read at 550 nm. The percentage of inhibition of the ferrozine -Fe^{2+} complex formation was calculated as follows:

Chelating ability % = [($A_{Control}$ _ A_{Sample} / $A_{Control}$) × 100]

A lower absorbance indicated higher chelating ability. The extract concentration producing 50% chelating ability (EC_{50}) was calculated from the curve for antioxidant activity percentage plotted the extract concentration. EDTA used as the positive control.

Superoxide Radical Scavenging Assay: The scavenging activities of *Termitomycetes* species were determined by

the nitro-blue tetrazolium (NBT) reduction method. In this method, O_{2-} generated *in vitro*, by xanthine oxidase, reduced the yellow dye (NBT^{2+}) to produce the blue formation which was measured spectrophotometrically at 560 nm against a blank [8]. Samples and positive controls were added to the reaction mixture in which O_2^- was scavenged thereby, inhibiting the NBT reduction. BHT was used as the positive control. The superoxide anion scavenging activity was calculated according to the following equation:

Superoxide anion radical-scavenging activity(%)=$[(A_0-A_1)/A_0]\times100$

Where, A_0 is the absorbance without samples and A_1 the absorbance in the presence of the extracts.

EC_{50} value (mg extract/mL) is the effective concentration at which superoxide anion radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis.

Hydroxy Radical Scavenging Effect: The hydroxy radical scavenging effect was assayed according to the method of Rajeshwar *et al*. [9]. The reaction mixture contains deoxyribose (2.8mM), $FeCl_3$ (0.1mM), EDTA (0.1mM), H_2O_2(1mM), ascorbate (0.1mM), KH_2PO_4- KOH buffer (20mM pH 7.4) and various concentration of sample extracts in final volume of 1.0 mL. The reaction mixture was incubated for 1 hr. at 37°C. The extent of deoxyribose degradation was measured by TBA method. 1.0 mL of TBA 1% (w/v) were added to the mixture and heated in a water bath for 100°C for 20min. the absorbance of resulting solution was measured spectrophotometrically at 530nm. The inhibition of degradation was calculated according to the equation

I = A_0-A_1/A_0x100,

Where, A_0 is the absorbance of the control reaction, A_1 is the absorbance of test compound.

Determination of Antioxidant Compounds: The content of total phenolic compounds in dry mushroom extracts was determined by Folin-Ciocalteu procedure [10] using Gallic acid as a standard. Absorbance was measured at 765 nm. Content of total phenolic compounds has been expressed as mg of Gallic acid equivalent (GAE) per g of dry mushroom extract.

The total flavonoids content has been determined by aluminium chloride colorimetric assay [11] using

Catehin as a standard. It has been expressed as mg of catehin equivalents (CE) per g of dry mushroom extract (mg CE/g).

Ascorbic acid was determined according as per the method of Klein and Perry [12]. The dried extract (100 mg) was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and was filtered through disposable membrane filter (0.45 µm, Millipore). The filtrate (1 mL) was mixed with 9 mL of 2, 6-dichlorophenolindopkenol and the absorbance was read at 515 nm against a blank. Content of ascorbic acid was estimated on the basis of the calibration curve of authentic L-ascorbic acid. The result was expressed as mg of ascorbic acid/g of the extract.

β- Carotene and lycopene were determined according to the method of Nagata and Yamashita [13]. The dried extract (100 mg) was vigorously shaken with 10 mL of the acetone hexane mixture (4:6) and was filtered through a membrane filter (0.45 µm Millipore). The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene and lycopene were estimated according to the following equations:

- Lycopene (mg/100mL) = $-0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$.
- β-carotene ((mg/100mL) = $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$.
- The results were expressed as mg of carotenoid/g of extracts.

RESULTS

The antioxidant activities of methanolic extract of the *T. heimii* and *T. microcarpus* were studied. The total antioxidant activity of the extracts were studied by the following standard methods such as, ABTS$^+$ Radical scavenging activity, DPPH radical scavenging activity, Reducing power assay, Chelating effects on ferrous ions, Superoxide radical scavenging activity, Nitric oxide radical scavenging activity, hydroxyl radical scavenging activity and determination of antioxidant compound.

The scavenging efficacy of both the extracts against DPPH radical increased with the increase in concentration. Elevated scavenging activity 67.36% was observed with the *T. heimii*, while 55.1% occurred at *T. microcarpus* at 200 µg/mL concentration (Fig. 1).
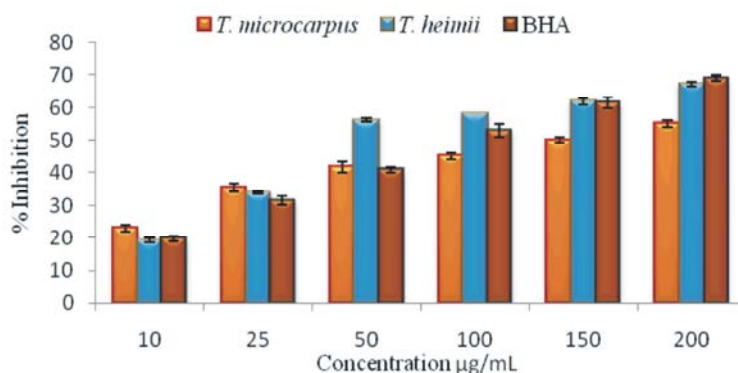

Fig. 1: DPPH radical scavenging activity of *T. microcarpus* and *T. heimii* Values expressed are means± SD (n=3) by three independent experiments.
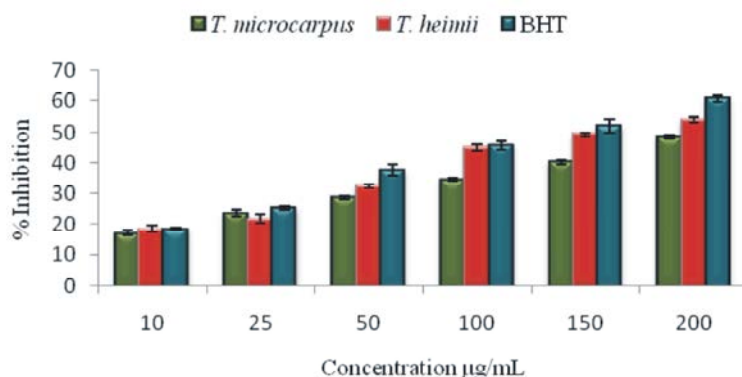

Fig. 2: ABTS$^+$ radical scavenging activity of *T. microcarpus* and *T. heimii* Values expressed are means± SD (n=3) by three independent experiments.
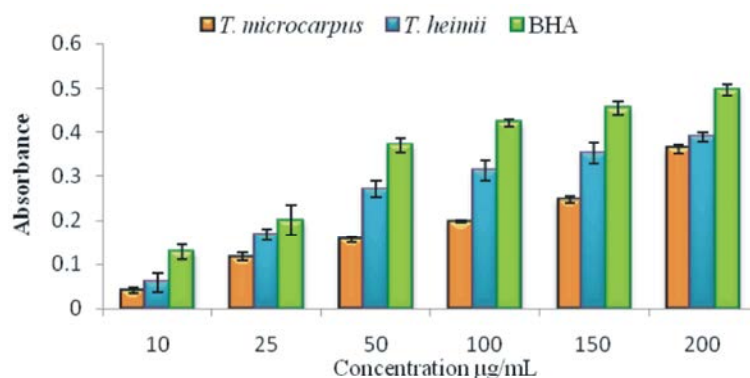
Fig. 3: Reducing power assay of *T. microcarpus* and *T. heimii*. Values expressed are means± SD (n=3) by three independent experiments.

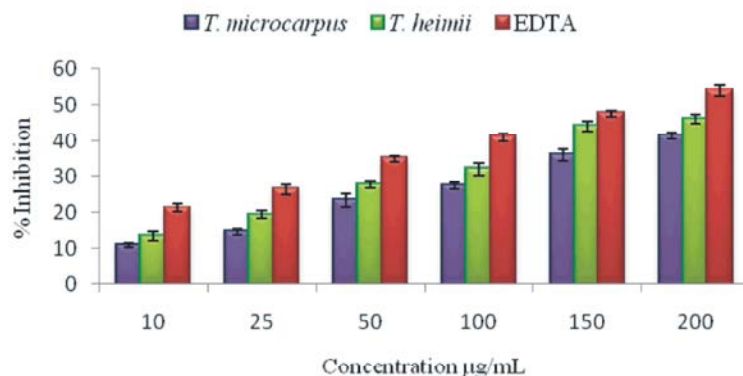Fig. 4: Ferrous ion chelating activity of *T. microcarpus* and *T. heimii*. Values expressed are means± SD (n=3) by three independent experiments.

The radical scavenging effect of both the extract against the ABTS$^+$ radical rose with increase in its concentration of extracts. In relation to both the extracts BHT displayed superior activity up to 200µg/mL. At 200µg/mL, the reduction was noticeable with 53.98 in *T. heimii* and 48.46% in *T. microcarpus*, respectively. The radical scavenging activity and the EC50 values against ABTS were better in *T. heimii* than the *T. microcarpus* (Fig. 2).

The reducing power of methanolic extracts of *T. heimii* and *T. microcarpus* are given in Fig. 3. The reducing power of both the extracts increased with an increase in concentration from 25 to 200µg/mL. The reducing power of *T. heimii* and *T. microcarpus* was found to be 0.388 and 0.362 at the concentration at 200µg/mL. However, the scavenging effect of BHA 0.496 (at 200µg/mL) was higher than the methanolic extracts of both the extracts.

In this assay, the chelating agents of the extracts disrupted the Ferrozine -Fe^{2+} complex thus lowering the intensity of the red colour. The chelating abilities of the methanolic extracts of *T. heimii* showed 46.2% and

T. microcarpus showed 41.51% at the concentration of 200µg/mL (Fig. 4). However, the chelating effect of EDTA showed more activity compared to both the extracts respectively.

The antioxidant activity of the mushroom extracts as measured by bleaching of β- carotene-linoleate system is given in Fig 5. The absorbance fell rapidly in samples without antioxidants whereas, in the presence of an antioxidant, they retained their colour and thus the absorbance for a longer time. The methanolic extracts of *T. heimii* (63.67%) show effective lipid peroxidation inhibition than *T. microcapus* (53.05%) and their EC$_{50}$ values 150 and 200 µg/mL respectively (Fig. 5). The methanolic extracts of *T. heimii* showed the strongest linoleic acid inhibition than the synthetic antioxidant BHA (55.24%).

The superoxide radical is known to be deleterious to cellular components as a precursor of more reactive oxygen species such as single oxygen and hydroxyl radicals. The results are presented in Fig. 6, which shows that the two extracts exhibited dose-dependent superoxide radical-scavenging activities.
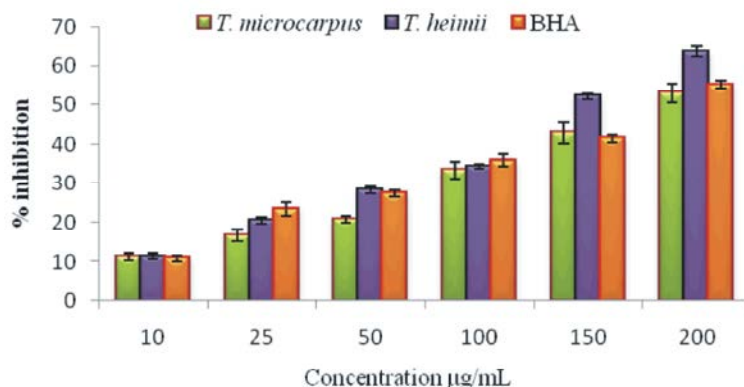
Fig. 5: β- Carotene linoleic acid activity of *T. microcarpus* and *T. heimii.* Values expressed are means± SD (n=3) by three independent experiments.
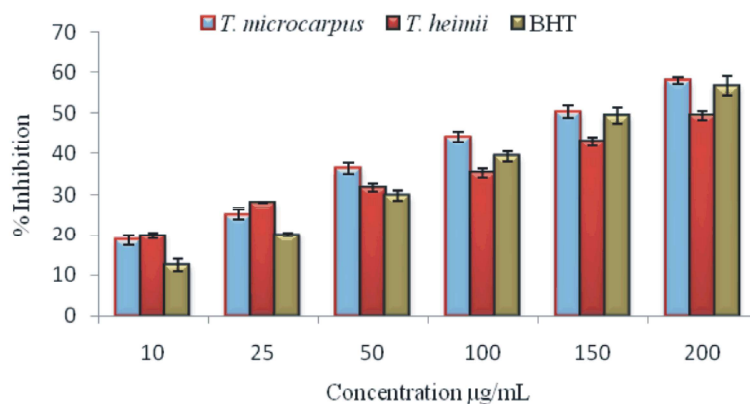

Fig. 6: Superoxide radical-scavenging activity of *T. microcarpus* and *T. heimii.* Values expressed are means± SD (n=3) by three independent experiments.
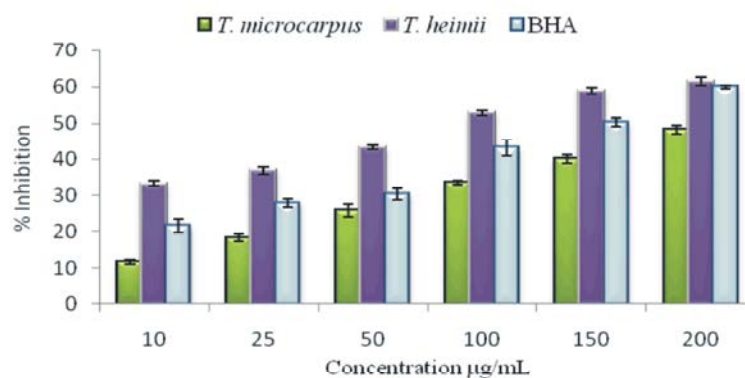

Fig. 7: Hydroxyl radical scavenging activity of *T. heimii* and *T. microcarpus.* Values expressed are means± SD (n=3) by three independent experiments.

The most potent activity was observed with the methanolic extract of *T. microcarpus* (57.9%). BHT acts as the positive control and showed 56.71% scavenging activity.

In the present study, the hydroxyl radical scavenging activity of the *T. microcarpus* and *T. heimii* were studied in six different concentrations (10, 25, 50, 100, 150, 200 µg/mL) of the extracts tested. BHA was used as the reference for hydroxyl radical. At 200µg/mL, the hydroxyl radical scavenging activity was the higher with 61.55% for *T. heimii* and 48.25% for the *T. microcarpus.* At the concentration of 10µg/mL, very low activity was observed both the extracts and the standards (Fig. 7).

Table 1: Antioxidant compounds from *T. microcarpus* and *T. heimii*.

Compounds	*T. microcarpus*	*T. heimii*
Phenols	10.47±0.96	12.26±0.72
Flavonoids	2.14±0.55	2.81±0.42
β- Carotene	0.2±0.03	0.25±0.07
Lycopene	0.37±0.03	0.25±0.02
Ascorbic acids	1.06±0.09	1.31±0.2

Values expressed are means± SD (n=3) by three independent experiments.

The naturally occurring antioxidant components, including total phenol, flavonoids, ascorbic acid, β-carotene and lycopene were found in both the extracts (Table 1). Total phenols were the major antioxidant compound detected in methanolic extracts. In the present study, However, the test fungus *T. microcarpus* and *T. heimii* seemed to store more antioxidant compounds (phenol 10.47 and 12.26, flavonoids 2.14 and 2.81, β-carotene 0.2 and 0.25, lycopene 0.37 and 0.25, ascorbic acid 1.06 and 1.31 mg/g).

DISCUSSION

The methanolic extracts of mushroom were subjected to screening for their possible antioxidant activity. Six complementary test systems, namely DPPH free radical scavenging, ABTS radical scavenging, β-carotene/linoleic acid systems, Super oxide dismutase, hydroxyl radical scavenging activity, reducing power assay, total phenolic compounds and total flavonoid concentration have been used for the analysis.

DPPH a stable free radical with a characteristic absorption at 517 nm was used to study the radical scavenging effect of extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay are shown in Fig. 1. The methanolic extracts of *T. heimii* and *T. microcarpus* showed a strong activity against DPPH radicals. These findings are similar to those explained by Sarikrukcu *et al*. [14] who found that the radical scavenging activity of the wild edible mushrooms such as *Lactarius deterrimus*, *Suillus collitinus*, *Boletus edulis* collected from Turkey were relatively high. The scavenging effect of methanolic extracts from *T. gibbosa* showed 91.47% at the concentration of 1 mg/mL [15] but in *T. microcarpus* and *T. heimii* showed 55.1% and 67.36% at the concentration of 200μg/mL respectively. These results indicated that methanolic extract of mushroom species have a noticeable effect on

scavenging free radical. However, the scavenging effects of BHA are higher than all methanolic extracts of mushroom species (Fig. 1). Similar results are also reported by Mau *et al*. [16].

ABTS, a stable free radical with a characteristic absorption peak at 734 nm was used to assess the radical scavenging efficacy of the extracts. The results demonstrated that extracts reacted with ABTS at different concentrations reduced the radical cation generated by the $ABTS^+$. The scavenging effects of both extracts against the ABTS radical rose up with an increase in its concentration from 25 to 200 μg/mL. The extent of reduction or decolourisation is directly proportional to the concentration of the antioxidant in question. However, even at 40 μg/mL concentrations the methanol extract of *M. conica* exhibited the highest radical scavenging activity (78.66%) when reacted with the $ABTS^+$ radicals [3]. *A. brasiliensis* showed 84% of the radical scavenging activity and the EC_{50} value was of 2.0 mg/mL [17] but even at lower concentration (EC_{50} at 0.6 mg/mL) the value was achieved and also its antioxidant efficacy was better than that of many other edible mushrooms.

Fe^{3+} reduction is often used as an indicator of the electron-donating activity. In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} and Fe^{2+} ions by donating the electron. This reducing capacity of compounds could serve as an indicator of potential antioxidant properties and the increasing absorbance suggests an increase in reducing power [18]. In the present study, *T. heimii* and the *T. microcarpus* contained the higher amount of reductant of 0.388 and 0.362 at the concentration of 200μg/mL in both extract respectively. However, the scavenging effect of BHA 0.496 was higher than the methanolic extracts of the samples. Similar results were reported from different mushroom extracts like *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albinomyces* [16]. The reducing power of methanolic extracts of *P. ferulae* ranged from 0.50 to 1.62 at 1.0-0.8 mg/mL concentration [19].

The free radical linoleic acid attacks the highly unsaturated β-carotene and the presence of different antioxidants can higher the extent of β-carotene-bleaching by neutralizing the linoleate free radical and the other free radicals formed in the system. By the β-carotene/linoleic acid method, the methanolic extracts of the *T. microcarpus* and *T. heimii* showed different patterns of antioxidant activities. The methanolic extract of *Morcella esculenta* and *M. angusticeps* show higher

β-carotene/linoleic acid activity (96.89 and 96.88%) at 4.5 mg/mL concentration [3]. But *T. heimii* methanolic extract showed 63.67% activity at 200 μg/mL concentration. Barros *et al*. [20] reported that the antioxidant activities of the various extracts of *Leucopaxillus giganteus*, *Sarcodon imbricatus* and *Agaricus arvensis* rose with increasing concentration and their activities were 61.4, 54.3 and 46.7% at 5 mg/mL concentration.

Superoxide is also known to indirectly induce lipid peroxidation as a result of H_2O_2 formation, creating precursors of the hydroxyl radicals [21]. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Methanolic extract of *T. microcarpus* proved to be better at scavenged O_2^- than *T. heimii*. This may be explained by the interaction of the different flavonoids in these extracts [22]. The mushroom species have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity. The percentage inhibition of superoxide generation by 200 μg/mL concentration of methanolic extract of wild edible mushroom species was found as 57.9 and 49.27%, respectively. The ethanolic extracts of *Tuber indicum* scavenged superoxide radicals by 63.3% at a concentration of 10 mg/mL [23]. It is noticeable that the methanolic extract of the *Termitomycetes* species exhibited a better scavenging of superoxide radicals than many common edible mushrooms.

Ferrous ions, the most effective pro-oxidants are commonly found in food systems. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activities of the coexisting chelator [24]. The percentage of metal chelating capacity 200 μg/mL concentration of methanolic extract of *T. heimii*, *T. microcarpus* and BHA were found as 46.2, 41.51 and 54.11, respectively. Wong and Chye [25] revealed that the methanolic extract of the wild edible mushrooms were better chelator of ferrous ion than those of commercial mushrooms [26]. Since, ferrous ions were the most effective pro-oxidants in the food systems [27], the high ferrous ion chelating abilities of the methanolic extracts of both species prove beneficial to human health.

The hydroxyl radical is considered the most reactive free radical encountered by the biological systems. It readily reacts with macromolecules such as amino acids, proteins and DNA thus resulting in cell damage. It is also believed to be an active inducer of peroxidation of the lipids. The ethanolic extracts of *Hypsizygus marmoreus* did not elicit any scavenging ability at a concentration of 5-20 mg/mL [28]. For example, the percentage of hydroxyl radical scavenged were 50% and 56.2% at the concentration of 8 mg/mL and 10 mg/mL respectively. At 40 mg/mL methanolic extract from specialty mushrooms *Dictyophora indusiata* and *Tricholoma giganteum* scavenged the hydroxyl radical by 75 and 69.4% [29]. The results suggest that the hydroxyl radical-scavenging abilities of *T. heimii* and *T. microcarpus* are comparable to or even more effective than those of the other mushrooms mention earlier. They also indicate that the methanolic extracts of *Termitomycetes* species by exerting a scavenging effect on the hydroxyl radical could help prevent oxidative damage in the human body.

Edible mushrooms harbour in them certain potent antioxidant compounds such as phenolics, flavonoids, ascorbic acid and α-tocopherol [2, 30]. In the present study, phenol was the major antioxidant component found in all the extracts of Termitomycetes followed by flavonoids, β carotene and lycopene that were found in scanty amount (Table 1) and this is in agreement with the previous report on *Agaricus* species [31]. Besides phenols, flavonoids are also reported as free radical scavengers by disrupting the chain reaction during the oxidation of triglycerides [32].

In *Russula delica*, the antioxidant compounds such as β-carotene (0.11 mg/g), lycopene (0.03 mg/g), ascorbic acid (2.93 mg/g) and phenol (6.23 mg/g) were reported [33]. However, the test fungus *T. microcarpus* and *T. heimii* seemed to store more antioxidant compounds (phenol 10.47 and 12.26, flavonoids 2.14 and 2.81, β-carotene 0.2 and 0.25, lycopene 0.37and 0.25, ascorbic acid 1.06 and 1.31 mg/g) than *Russula delica*.

The highest content of total polyphenolics in *T. microcarpous* and *T. heimii* might be the key components accounting for the better results found in total antioxidant activity, reducing power, metal chelating activity and superoxide anion scavenging abilities as compared to other mushroom species. Numerous studies have conclusively showed that consumption of foods high in phenolic content can reduce the risk of heart diseases by slowing the progression of atherosclerosis, because they act as antioxidants [4]. Therefore, the results of the present study suggest that the extracts of *Termitomycetes* species as well as of the other edible mushrooms might mitigate the oxidative damage in the human body and offer health protection from the oxidative stress.

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