KAVAKA 54: 107-114 (2020) doi:10.36460/Kavaka/54/2020/107-114

Antioxidant Properties of Two Wild Edible Mushrooms from Andhra Pradesh

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(Submitted on May 2, 2020; Accepted on June 16, 2020)

ABSTRACT

Investigations on the antioxidant potential of the methanolic extract of two wild edible mushrooms, namely *Podaxis pistillaris* (L. ex Pers.) Fr. and *Termitomyces heimii* Natarajan from Anantapur city in the arid zone of Andhra Pradesh, India has been included in this manuscript. Different concentrations (0.25-1.25 mg/mL) of extract were used for the assays. Out of the two mushrooms, *Podaxis pistillaris* show very high antioxidant properties in terms of total phenolic content (93.08±2.02 mM GAEs/g at 1.25mg/mL), DPPH radical scavenging activity (91.69±0.15% at 1mg/mL), ABTS radical scavenging assay (98.53±1.50% at 1mg/mL), ferric reducing antioxidant power assay (36.50±0.13 µM trolox equivalents/mg at 1.25mg/mL) and ferrous-ion chelating activity (89.50±0.46% at 1.25mg/mL).

Keywords: *Podaxis pistillaris*, *Termitomyces heimii*, antioxidant activity, total phenolic content

INTRODUCTION

Antioxidants are known to possess radical scavenging capacity and to exert a potential protective effect against the free radical damage (Kris-Etherton *et al.,* 2002). In biological systems, antioxidants protect against oxidative damage and help in preventing cardiovascular, neurological and/or carcinogenic diseases (Lanina *et al.,* 2007). Antioxidants are considered as an important group of food additives that have the ability to protect against detrimental change of oxidized nutrients and hence they can be used to preserve or extend shelf-life of food (Andre *et al.,* 2010).

Mushrooms are one of the natural sources of antioxidants and also of beneficial nutraceutical products which add to the endogenous protective system (Jayakumar, 2011; Reis *et al.,* 2012; Atri *et al.*, 2016; Mridu and Atri, 2017; Kore, 2020). Therefore, the investigations of the antioxidants especially from natural sources like dietary foods, vegetative mushrooms and others is the need of the hour (Lakhanpal *et al.,* 2016; Acharya *et al.,* 2017; Atri *et al.,* 2019). Although numbers of cultivated and wild edible mushrooms were studied for their antioxidant properties from different geographic region of the world, this study is focused on mushrooms from arid region of Andhra Pradesh in India.

MATERIALS AND METHODS

Samples: The sporophores of two wild edible mushrooms, *Podaxis pistillaris* (L. ex Pers.) Fr.(**Fig.1**) and *Termitomyces heimii,* Natarajan (**Fig.2**) were collected after rainy season from the College Campus of Sri Sathya Sai Institute of Higher Learning at Anantapur, located in the semi-arid region of Andhra Pradesh, India. After collection the mushrooms were taxonomically worked out and identified after consulting the authentic literature (Natarajan, 1979; Atri *et al.*, 2005; Mridu and Atri, 2015). The sporophores were subsequently cleaned, sliced into thin pieces and dried in an oven at 40°C. Dried mushrooms were ground to a fine homogeneous powder using mortar and pestle and then powder was stored in an amber colored glass bottle at room temperature.

Sample preparation: Methanolic extract was prepared by sonicating and stirring 3g of mushroom powder in 80% methanol at 150 rpm for overnight at 25°C and then filtered through Whatman No.1 filter paper. The residue was re-extracted with two additional proportion of 50mL of methanol as described above. Filtrates were pooled, centrifuged and were evaporated at 40°C to dryness under reduced pressure using Heidolph Rotary evaporator. Extracts were stored at -20°C until used.

Standards and reagents: The standards: Gallic acid (PubChem CID: 370), Trolox (PubChem CID: 40634), Folin-Ciocalteu reagent (FCR), 2,2'-Azino-bis

Fig.1. *Podaxis pistillaris* Field Photograph

Fig. 2. *Termitomyces heimii* Field Photograph

(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Company. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), and ferrozine reagents were purchased from HiMedia Laboratories Private Limited. Sodium carbonate, disodium ethylenediaminetetraacetic acid (Na_2EDTA), sodium acetate, ferric chloride, ferrous sulphate, potassium persulphate, hydrochloric acid, methanol and 95% ethanol, all these chemicals were of analytical grade and purchased from Merck Life Sciences Private Limited.

Determination of total phenolic content: Total phenolic content in mushrooms was determined using slightly modified method of Slinkard and Singleton (1977) with gallic acid as a standard. To various concentrations of mushroom extracts Folin-Ciocalteu reagent was added and mixed thoroughly. The saturated sodium carbonate was added to the mixture, incubated for two hours and the absorbance was measured at 760nm. The reduction of folin reagent by the sample was expressed as mM of gallic acid equivalent (GAE) per mg of extract. The concentration of phenolic compounds was calculated according to the equation obtained from graph of standard gallic acid.

$$\text{Absorbance} = 14.80 * \text{Gallic acid} + 0.1720 \qquad (R^2 = 0.9936)$$

DPPH radical scavenging activity: The free radical scavenging activity was determined spectrophotometrically by the DPPH assay (Blois, 1958) with slight modifications. Mushroom extracts of different concentrations were used for antioxidant activity. To 500µL of sample extract, 1.5mL of 0.2mM DPPH was added and mixed thoroughly. Solution was kept for 30 min at room temperature and the absorbance was measured at 517nm. Methanol was used as control. The capability of scavenging the DPPH radical was calculated as percentage using the following formula:

$Abs_{control}$ is the initial concentration of the DPPH without sample and Abs_{sample} is the absorbance of the remaining concentration of DPPH in the presence of extracts.

$$\% \text{ Radical Scavenging Activity (RSA)} = \left[1 - \frac{Abs_{sample}}{Abs_{control}}\right] \text{X } 100 \quad \text{where,}$$

The extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage against extract concentration. Trolox was used as an antioxidant standard for comparison of the activity.

ABTS radical scavenging assay: The spectrophotometric determination of $ABTS^+$ scavenging activity was analyzed according to the method of Re *et al.* (1999), with slight modification. The $ABTS^+$ radical was prepared by mixing 7mM ABTS stock solution with 2.45mM potassium per sulphate (1/1, v/v) and the mixture was left for 12-16 hrs at room temperature. Before usage, the $ABTS^+$ radical solution was diluted with 95% ethanol (approx. 1:40) to obtain an absorbance of 0.7±0.02 units at 734nm. Ethanol was used a blank. Then, 1mL of $ABTS^+$ radical solution was added to 1mL of sample extracts of various concentrations and the absorbance was measured at 734nm. The scavenging capability of $ABTS^+$ was calculated using the following equation:

$$\% \text{ ABTA}^+ \text{ Scavenging Activity} = \left[1 - \frac{Abs_{sample}}{Abs_{control}}\right] \text{X } 100 \quad \text{where,}$$

Trolox was used as an antioxidant standard for comparison of the activity and was expressed as µM of Trolox Equivalent Antioxidant Capacity (TEAC) per mg of extract.

FRAP assay: The ferric reducing antioxidant power (FRAP) method is based (Benzie and Strain, 1996; Benzie and Szeto, 1999) on the reduction of a ferroin analog, the Fe^{3+} complex of tripyridyltriazine [$Fe(TPTZ)_{3+}$], to the intensely blue coloured Fe^{2+} complex $Fe(TPTZ)_{2+}$ by antioxidants in acidic medium. Antioxidant activity in mushroom samples was measured using modified method. The oxidant in this assay was prepared by mixing acetate buffer (pH 3.6), 10mM TPTZ in 40mM HCl and 20mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v) and was called as "FRAP reagent". Freshly prepared 1.5mL of FRAP reagent was added to 500µL of sample extracts of different concentrations and was vortexd thoroughly. Obtained mixture was stirred in shaking water bath at 37°C and absorbance was measured at 593nm. FRAP reagent was used as blank. Trolox was used to obtain the standard curve and the FRAP value of sample was expressed as µM of trolox equivalent per mg of extract. The FRAP value of sample in micro molar was calculated using the following formula:

$$\text{FRAP value of sample } (\mu M) = \frac{Abs \text{ (sample)} \; X \; FRAP \; value \; of \; std. \; (\mu m)}{Abs \text{ (std.)}}$$

Ferrous-ion chelating activity: Ferrous chelating ability (FCA) in mushrooms was determined using modified method of Dinis *et al.* (1994). To the mushroom extract of various concentrations 100µL of 2mM $FeSO_4$ was added and mixed thoroughly. The reaction was then initiated by the addition of 100µL of 5mM Ferrozine. The solution was left at room

temperature for 10 min and absorbance was measured at 562nm. The inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated by using the formula given below:

$$\% \text{ Ferrous Chelating Ability} = \left[1 - \frac{Abs_{sample}}{Abs_{control}}\right] X\ 100 \quad \text{where,}$$

$Abs_{control}$ is the absorbance of complex formation molecules which is magenta in colour and Abs_{sample} is the absorbance of the sample extract. EDTA was used as a standard. EC_{50} value (mg extract/mL) is the effective concentration at which ferrous ion were chelated by 50% and was obtained by interpolation from linear regression analysis.

Statistical analysis: Each sample was assayed with three replicates and all the assays were performed in triplicate. The results were expressed as mean ± standard deviation (n=3). The experiment data was subjected to one-way analysis of variance (ANOVA) for completely randomized design to determine the least significant difference amongst mean at the 5% level of significance.

RESULTS AND DISCUSSION

Extraction yield: Solubility of antioxidants is usually affected by the polarity of the solvent(s) used. Solvents such as methanol, ethanol, acetone, ethyl acetate, water and their combinations have also been used for extraction of antioxidant compounds. In the present study methanol was used as extraction solvent. The yield obtained from methanolic extracts of two edible wild mushrooms varied considerably. The highest yield was obtained from *Podaxis pistillaris* extract (38.44 ± 1.57 % w/w) followed by the extract of *Termitomyces heimii* (34.44 ± 1.92% w/w).

Total phenolic content: Total polyphenols are the major naturally occurring antioxidant components found in wild edible mushrooms. In this study, the total phenolic content of mushrooms extract was tested using the concentrations ranging from 0.25 - 1.25mg/mL. The results obtained are expressed in mM GAEs/g of dry mushroom extract (**Table 1**). The value of phenolic compounds found in methanolic extract of *Podaxis pistillaris* and *Termitomyces heimii* were 83.55 ± 3.72 and 24.84 ± 3.50 mM GAEs/g, respectively at 1mg/mL concentration.

As compared the total phenolic content reported in ethanolic and aqueous extracts of *Pleurotus sajor-caju* was 9.48±0.85 and 10.4±1.37 mg GAEs/g, respectively at 16mg/mL concentration (Kanagasabapathy *et al*., 2011) and in case of *Pleurotus ostreatus* it is reported to be 30.93 ± 1.92 and 42.47 ± 2.27 mg GAEs/g, respectively (Chirinang and Intarapichet, 2009). At 10mg/mL concentration, the aqueous extract of *Pleurotus porrigens* is reported to contain 5.04 ± 41.18 mg GAEs/g phenolic content (Yim *et al*., 2012).

In the methanolic extract of *Podaxis pistillaris* (at 1.25mg/L) evaluated presently very high phenolic content (93.08 ±2.02 mM GAEs/g) has been documented as compared to the reported values of phenolic content in the ethanolic extract of *Trametes versicolor* (9.58±1.59 mg GAEs/g), *Polyporus fomentarius* (47.29±1.37 mg GAEs/g), *Cantharellus cibarius* (31.48±1.57 mg GAEs/g) and *Lactarius deliciosus* (51.27±1.44 mg GAEs/g) by Orhan and Üstün (2011). Similarly Arbaayah and Umi (2013) evaluated 40.51 ± 0.72 mg Tannic Acid Equivalents (TAEs)/g DW in *Schizophyllum commune*, 51.94±0.67 mg TAEs/g in *Pleurotus djamor* var. *djamor* and 39.36±0.19 mg TAEs/g in *Pleurotus pulmonarius*. In the methanolic extract of *Agaricus bisporus* (85.45±0.36 mg Pyrocatechol Equivalents (PEs)/g)), *A.bitorquis* (33.15±0.10 mg PEs/g) and also in *A.essettei* (53.45±0.20 mg PEs/g) much less amount of phenolic content has been evaluated by Ozturk *et al.* (2011) in comparison to the net amount of this component evaluated in *Podaxis pistillaris* during the present investigations.

DPPH radical scavenging activity: The method of scavenging "stable" DPPH free radical can be used to evaluate the antioxidant activity of extracts or specific compounds. The per cent scavenging activity of DPPH radical observed presently using methanolic extracts of mushrooms at various concentrations is given in tabulated form (**Table 2**). As is evident from the values obtained the radical scavenging activity of the methanolic extracts of *Podaxis pistillaris* was found to be higher in comparison to the scavenging activity of the methanolic extracts of *Termitomyces heimii*. At 1mg/mL, *Podaxis pistillaris* exhibited very high radical scavenging activity (91.69 ± 0.15%) which is significantly higher in comparison to the number of such reports from elsewhere on other mushrooms. Sarikurkcu *et al.* (2010) while working with *Amanita caesarea* (79.4±1.4%), *Clitocybe geotrope* (64.8±1.9%) and *Leucoagaricus pudicus* (64.6±0.8%) reported much less scavenging activity when -20 mg/mL methanolic extracts of these mushrooms was used for evaluation. Similarly Orhan and Üstün (2011) also reported lower value of the scavenging activity of ethanolic extracts (5mg/mL) of *Cantharellus*

Table 1. Concentrations of total phenolics (mM GAEs/g) in methanolic extracts.

Sample	Sample ID	Total phenolics[a] (mM GAEs[b]/g of extract)				
		Sample Concentration (mg/ml)				
		0.25	0.50	0.75	1.00	1.25
Podaxis pistillaris	PP	17.71±1.16	41.95±2.16	65.77±2.41	83.55±3.72	93.08±2.02
Termitomyces heimii	TH	0.00 ± 0.41	07.18±1.54	16.49±2.69	24.84±3.50	30.38±3.56

[a] Each value is expressed as mean ± standard deviation ($n = 3$).
[b] GAEs, gallic acid equivalents.

Table 2. DPPH radical scavenging activity (%) of the methanolic extracts of mushrooms at different concentrations[a]

Sample	Sample ID	Sample Concentration (mg/ml)				
		0.25	0.50	0.75	1.00	1.25
Podaxis pistillaris	PP	34.07±2.57	66.40±4.42	90.28±1.03	91.69±0.15	91.45±0.38
Termitomyces heimii	TH	7.15±1.26	11.43±1.85	16.07±2.23	21.15±3.60	23.27±4.37
		Standard Concentration (μM)				
Standard		12.5	25.0	37.5	50.0	62.5
Trolox[b]		15.97±0.83	33.84±1.20	49.30±1.52	69.87±0.72	88.96±1.66

[a] Each value is expressed as mean ± standard deviation ($n = 3$).
[b] Trolox, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

cibarius (59.87±2.34%), *Lactarius deliciosus* (62.41±0.67%), *Trametes versicolor* (28.69±0.50%) and *Polyporus volvatus* (84.29±0.67%). However, in comparison the radical scavenging activity of methanolic extracts (300mg/mL) of stipe (85.5%) and pileus (84.49%) of *Russula griseocarnosa* is not exactly the same but almost near to the scavenging activity of the methanolic extract of the presently evaluated mushrooms (Chen *et al.*, 2010). Kalyoncu *et al.* (2010) also evaluated the ethanolic extracts (1mg/mL) of *Omphalotus olearius* (60.25%), *Morchella esculenta* (27.41%), *Ganoderma lucidum* (10.75%) and *Lentinula edodes* (6.20%) and documented very low percentage of radical scavenging activity in comparison to the presently evaluated mushrooms.

In present study, lower DPPH EC_{50} values (**Table 6**) for *Podaxis pistillaris* (0.486 mg/mL) indicates that this mushroom have better antioxidant properties than the other mushrooms. EC_{50} values in scavenging the DPPH radical for methanolic extract of stipe (1.93±0.03 mg/mL), cap (1.77±0.03 mg/mL) and gills (0.82±0.02 mg/mL) of *Agaricus bisporus* as reported by Savoie *et al.* (2008) is much less in comparison to DPPH EC_{50} values obtained for *Podaxis pistillaris* during the present investigations. Similarly, Ozturk *et al.* (2011) also reported lower EC_{50} values of DPPH scavenging effect obtained from methanolic extract for *Agaricus bisporus* (0.988±0.13 mg/mL), *A. bitorquis* (0.590±0.31 mg/mL) and *A. essettei* (0.921±0.07 mg/mL) when compared with the similar such observations made presently while working with the two wild edible mushrooms.

ABTS radical scavenging assay: Antioxidant activities using the sporophores of *Podaxis pistillaris* and *Termitomyces heimii* determined as per cent scavenging activity of $ABTS^+$ radical are presented in **Table 3**. As is apparent from the generated data given in the table, at 1.25mg/mL concentration, the methanolic extract of *Podaxis pistilaris* exhibited highest RSA (98.83±0.91%) when reacted with $ABTS^+$ radical in comparison to *Termitomyces heimii* (61.00±9.41 %).

During present study, the evaluated per cent scavenging activity of *Podaxis pistillaris* at 1mg/mL concentration is significantly higher (98.53±1.50%) when compared with the $ABTS^+$ radical scavenging activities reported when the ethanolic extract of *Omphalotus olearius* (88.01%), *Morchella esculenta* (87.07%), *Ganoderma lucidum* (22.28%) and *Lentinula edodes* (43.00 %) was used (Kalyoncu *et al.*, 2010). In the ethanolic extract of *Pleurotus tuber-regium* at 5mg/mL concentration Lin *et al*., (2014) reported 13.54±2.51% $ABTS^+$ radical scavenging activity which is much less in comparison to what has been obtained during the present investigation while working with *Podaxis pistillaris* and *Termitomyces heimii*. In aqueous extract of *Pleurotus porrigens* 77.48±5.02 % $ABTS^+$ radical scavenging activity has been documented by Yim *et al.* (2012) which is much on the higher side in comparison to what has been reported by Lin *et al*., (2014) in the ethanolic extract of *Pleurotus tuber-regium* but it is still less in comparison to $ABTS^+$ radical scavenging activity of methanolic extract of *Podaxis pistillaris* evaluated presently.

The $ABTS^+$ radical scavenging activity of methanolic extract is also expressed as Trolox Equivalent Antioxidant Capacity (TEAC) and defined as the μmol of Trolox equivalents (TEs) per milligram of mushroom extract. In terms of TEAC, the *Podaxis pistillaris* and *Termitomyces heimii* revealed highest

Table 3. $ABTS^+$ radical scavenging activity (%) of the methanol extracts of mushrooms at different concentrations[a]

Sample	Sample ID	Sample Concentration (mg/ml)				
		0.25	0.50	0.75	1.00	1.25
Podaxis pistillaris	PP	39.14±4.04	70.36±2.18	89.97±1.87	98.53±1.50	98.83±0.91
Termitomyces heimii	TH	16.81±5.36	27.20±7.50	43.55±7.64	52.76±9.18	61.00±9.41
		Standard Concentration (μM)				
Standard		1.875	3.750	5.625	7.500	9.375
Trolox[b]		12.36±2.44	28.15±0.88	52.32±2.71	75.28±1.82	80.57±1.16

[a] Each value is expressed as mean ± standard deviation ($n = 3$).
[b] Trolox, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

value i.e., 0.550 and 0.561 µmol TEs/mg, respectively. Kanagasabapathy *et al.* (2011) while working with the aqueous and ethanolic extract of *Pleurotus sajor-caju* evaluated the scavenging activity at 29.45±3.07 and 16.58± 3.09 µmol TEs/g, respectively which is much on the lower side in comparison to its value documented in the methanolic extract of *Podaxis pistillaris* and *Termitomyces heimii*. In the ethanolic extract of *Lentinula edodes* also Choi *et al.* (2006) evaluated 7.6 mg Ascorbic acid Equivalents (AEs)/100g scavenging activity when treated at 100°C and 10.8 mg AEs/100g scavenging activity when treated at 121°C for 30min which is quite less in comparison to the presently evaluated mushrooms.

In the data presented in **table 6**, the antioxidant properties have been expressed as the EC_{50} values for reducing power, DPPH, ABTS scavenging effects and ferrous chelating ability obtained from methanolic extracts of each mushroom. The effectiveness of antioxidant properties is inversely correlated with their EC_{50} values. While working with *Agaricus bisporus*, Savoie *et al.* (2008) evaluated the EC_{50} values in scavenging the activity of ABTS radical in the methanolic extract of stipe (2.95±0.08 mg/mL), cap (2.90±0.11 mg/mL) and gills (1.16±0.02 mg/mL) separately. Ozturk *et al.* (2011) also evaluated the EC_{50} values of ABTS scavenging effect obtained from methanolic extract for *Agaricus bisporus* (0.241±0.07 mg/mL), *A. bitorquis* (0.158±0.03 mg/mL) and *A. essettei* (0.347±1.02 mg/mL). As compared during the present study, lower ABTS EC_{50} values were obtained for *Podaxis pistillaris* (61.57µg/mL) and *Termitomyces heimii* (94.78µg/mL) which clearly indicates that these mushrooms have better antioxidant properties in comparison to *Agaricus bisporus, A. bitorquis* and *A. essettei* .

FRAP assay: The reducing power (FRAP) assay, using methanolic extracts of *Podaxis pistillaris* and *Termitomyces heimii* in different concentrations ranging from 0.25-1.25 mg/mL, when performed, the FRAP value was found to increase with the increasing concentration of the extracts used (**Table 4**). The extract of *Podaxis pistillaris* gave 35.02±0.20 µM TEs/mg reducing power which is much higher than 12.13±2.07 µM TEs/mg reducing power measured with the methanolic extract of *Termitomyces heimii* at a concentration of 1mg/mL.

During the present study, the mushroom sample of *Podaxis pistillaris* from Anantapur displayed high ferric reducing antioxidant power (36.50±0.13 µM TEs/mg) at 1.25mg/mL concentration compared to the FRAP values evaluated in number of mushrooms except *Suillus luteus* (53.52 µM/mg). Keles *et al.* (2011) evaluated the reducing power of methanolic extract of *Agaricus bisporus* (12.17 µmol/mg), *Pleurotus ostreatus* (2.38 µmol/mg), *Hydnum repandum* (0.14 µmol/mg), *Lactarius deliciosus* (2.67 µmol/mg) and *Russula nigricans* (23.60 µmol/mg). The values reported for different evaluated mushroom samples were substantially on the lower side in comparison to the presently obtained values. Even the methanolic extract of *Lactarius sanguifluus* (2.12±0.46 µmol TEs/100g), *Russula delica* (1.18±0.30 µmol TEs/100g) and *Suillus bellinii* (4.54±0.16 µmol TEs/100g) exhibited much lower FW FRAP values (Kalogeropoulos *et al.*, 2013). Reducing power assay of ethanolic and aqueous extracts (16mg/ml) of *Pleurotus sajor-caju* was evaluated as 26.29 ± 2.82 and 35.06 ± 0.86 µmol FEs/g (Kanagasabapathy *et al*., 2011) and that of *Pleurotus ostreatus* was evaluated (20mg/mL) as 1.61 and 4.38 µmol Fe^{2+}/mL, respectively (Chirinang and Intarapichet, 2009). Yim *et al.* (2012) reported 6.71 ± 0.61 µmol FEs/100g FRAP assay in the aqueous extract of *Pleurotus porrigens*.

Ferrous-ion chelating activity: In this assay, the chelating agents disrupt the Ferrozine-Fe^{2+} complex, thereby, decreasing the intensity of purple colour. The rate of colour reduction which is measured at 550nm is used for the estimation of the chelating activity. A lower absorbance indicates high chelating ability.

The chelating ability of the methanolic extracts of *Podaxis pistillaris* was found to be higher than the chelating ability of the methanolic extract of *Termitomyces heimii* (**Table 5**). At 1.25mg/mL, the methanolic extracts of *Podaxis pistillaris* and *Termitomyces heimii* was found to chelate 89.50±0.46% and 81.43±1.90% ferrous-ions, respectively. Ferrous chelating ability of both the mushrooms has been found to be relatively higher when compared to the chelating ability of some of the mushrooms evaluated in this regard. The ferrous chelating ability when methanolic extract was used at 10mg/mL concentration has been reported at 62±7.8% in *Agaricus blazei* (Soares *et al.*, 2009) and 60.68% in *Pleurotus*

Table 4. FRAP value (µM Trolox Equivalents/mg) of the methanolic extracts of mushrooms at different concentrations[a] using FRAP assay.'

Sample	Sample ID	FRAP Value[a] (µM TEs[b]/mg of extract)				
		Sample Concentration (mg/ml)				
		0.25	0.50	0.75	1.00	1.25
Podaxis pistillaris	PP	12.89±0.40	23.28±0.76	31.20±0.43	35.02±0.20	36.50±0.13
Termitomyces heimii	TH	3.27±0.65	6.33±1.34	9.63±1.94	12.13±2.07	13.97±2.42
		Standard Concentration (µM)				
Standard		1.875	3.750	5.625	7.500	9.375
Trolox[c] (*Abs*)		0.07±0.0	0.19±0.02	0.29±0.0	0.38±0.0	0.45±0.01

[a] Each value is expressed as mean ± standard deviation ($n = 3$).
[b] TEs, Trolox equivalents.
[c] Trolox, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

Table 5. Metal chelating ability (%) of the methanolic extracts of mushrooms at different concentrations[a]

Sample	Sample ID	Sample Concentration (mg/ml)				
		0.25	0.50	0.75	1.00	1.25
Podaxis pistillaris	PP	60.61±2.67	78.08±1.77	82.75±0.89	87.51±0.29	89.50±0.46
Termitomyces heimii	TH	42.35±1.53	64.57±4.57	72.49±1.08	78.91±0.63	81.43±1.90
		Standard Concentration (μM)				
Standard		100	125	150	175	200
EDTA[b]		24.66±0.69	49.32±1.43	61.56±0.60	86.00±0.45	98.49±0.85

[a] Each value is expressed as mean ± standard deviation ($n = 3$).
[b] EDTA, ethylenediaminetetraacetic acid.

ostreatus (Jayakumar *et al.*, 2009). In comparison when 1mg/mL concentration of the methanolic extract was used the ferrous ion chelating ability has been documented at 60.1±2.1% in *Amanita caesarea*, 28.0±0.2% in *Clitocybe geotrope* and 88.0±1.1% in *Leucoagaricus pudicus* (Sarikurkcu *et al.*, 2010).

Orhan and Üstün (2011) also investigated the chelating abilities of ethanolic extracts of *Polyporus radiatus* (18.05±1.32%), *Lactarius deliciosus* (33.30±0.54%) and *Polyporus badius* (23.22±0.04%) using 5mg/mL concentration in the extract. Similarly methanolic extracts of stipe and pileus of *Russula griseocarnosa* has been reported to chelate 74.4% and 79.2% of ferric ions, respectively when at 10mg/mL concentration was used in the extract (Chen *et al.*, 2010). Kalogeropoulos *et al.* (2013) while evaluating the methanolic extracts of *Lactarius deliciosus* (52.3±2.8 μmol Fe^{2+}/100g), *L. sanguifluus* (49.8±5.7 μmol Fe^{2+}/100g), *L. semisanguifluus* (41.0±6.9 μmol Fe^{2+}/100g), *Russula delica* (52.5±8.3 μmol Fe^{2+}/100g) and *Suillus bellinii* (27.1±4.3 μmol Fe^{2+}/100g) documented the respective FW chelating values mentioned in parenthesis against the respective mushroom.

Presently chelated EC_{50} values for *Podaxis pistillaris* and *Termitomyces heimii* were evaluated at 0.95 and 0.98 mg/mL. As compared Oke and Aslim (2011) reported the EC_{50} values of chelating abilities obtained from methanolic extracts for *Pleurotus eryngii* and *Auricularia auricula-judae* to be 0.42±0.03 and 1.52±0.14 mg/mL, respectively.

CONCLUSION

The results presented on the antioxidant activities of *Podaxis pistillaris* and *Termitomyces heimii* and the comparison of the results made with similar such findings in the literature it becomes clear that in comparison to the other mushrooms, *P. pistillaris* possesses significant antioxidant properties in terms of total phenolic content, scavenging of radicals, chelation of ferrous-ions and for reducing power assay. As the presence of total phenols is the main component responsible for the "antioxidant power" of the mushrooms, it was found that *P. pistillaris* contained 93.08±2.02 μM gallic acid equivalents/gram of dry extract at 1.25mg/mL. The results of this study and the comparison of the results thereof indicate that the mushrooms are potential source of natural antioxidants and are best suited for use as dietary supplements, functional foods, and also for the development of nutraceuticals.

Table 6. Antioxidant activity in terms of EC_{50} values[a] (mg/ml or μg/ml) of methanolic extracts of wild edible mushrooms for DPPH, $ABTS^{+}$, FCA and FRAP assays.

Sample	DPPH Assay	$ABTS^{\bullet+}$ Assay	Ferrous chelating Assay (FCA)	FRAP Assay
	EC_{50} (mg/ml)	EC_{50} (μg/ml)	EC_{50} (mg/ml)	EC_{50} (mg/ml)
Podaxis pistillaris	0.486±.005	61.57±0.015	0.958±0.019	0.539±0.006
Termitomyces heimii	0.672±.031	94.78±0.039	0.982±0.018	0.656±0.029
Standard (in μM)	EC_{50} (μM/ml)	EC_{50} (μM/ml)	EC_{50} (μM/ml)	EC_{50} (μM/ml)
Trolox[b]	6.906±0.917	4.353±0.615	-	4.103±0.015
EDTA[b]	-	-	126.4±0.007	-

[a] EC_{50} values represent the means ± SE of three parallel measuring ($p<0.05$), the values written in bold show the highest activity.
[b] Reference compounds.

ACKNOWLEDGEMENTS

Authors would like to acknowledge UGC-SAP (DRS) and DST-FIST, Govt. of India for the infrastructural support to the Department of Biosciences, Sri Sathya Sai Institute of Higher Learning.

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