



## Nutritional Composition and Antimicrobial Activity of Two Wild Edible Mushrooms from Andhra Pradesh

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### ABSTRACT

The present work provides information on proximate composition, mineral element profile and antimicrobial activity of two wild edible mushrooms, namely, *Podaxis pistillaris* and *Termitomyces heimii* from Andhra Pradesh, India. Concentrations of four macro elements (Mg, K, P and Ca) and eight micro nutrient elements (Mn, Fe, Cu, Zn, Co, Se, B and Mo) were assessed using ICP-MS. *Termitomyces heimii* was found to possess high amount of magnesium, potassium, calcium, manganese, iron, zinc and cobalt. In *Podaxis pistillaris* as well good amount of copper, selenium and molybdenum were detected. Antimicrobial activities of mushroom samples when evaluated exhibited low to moderate activity against the normal and resistant strains of bacteria while the higher concentrations were not found to be effective.

**Key words:** Proximate composition, Mushrooms, Nutrient elements analysis, Antimicrobial activity

Wild edible mushroom has been traditionally used by people in the Orient countries since early times (Manzi *et al.* 1999, Sanmee *et al.* 2003, Ouzouni *et al.* 2007). The consumption of wild edible and cultivated mushrooms has increased during the recent years as they are now recognized as valuable source of health food, which is low in calories, rich in polysaccharides, essential amino acids, fibre, important vitamins and minerals (Atri *et al.* 2019, Lakhanpal *et al.* 2016, Heleno *et al.* 2010, Mendil *et al.* 2004, Ouzouni *et al.* 2004, Matila *et al.* 2002). Mushroom extracts and their phenolic compounds have been reported to exhibit antibacterial, antifungal, anti-inflammatory, antiviral and anticarcinogenic etc. (Alves *et al.* 2012, Soobrattee *et al.* 2005). In the present study the results on the evaluation of proximate components, chemical composition and antimicrobial activity of two wild edible mushroom species, namely, *Podaxis pistillaris* and *Termitomyces heimii* from Andhra Pradesh, India are being presented.

Two wild edible mushrooms, *Podaxis pistillaris* (L. ex Pers.) Fr. and *Termitomyces heimii* Natarajan were collected from Anantapur, Andhra Pradesh, India. These were taxonomically identified and authenticated based on their morphological and anatomical characteristics. The fruiting bodies of mushrooms were cleaned, sliced into thin pieces and were air-dried in an oven at 40°C. Dried mushrooms were further reduced to fine homogeneous powder and used for analysis.

#### Proximate composition

For proximate composition the ash content of samples was determined by incineration at 550°C (AOAC 1995), protein by Lowry method, fat content by soxhlet extraction method using petroleum ether as a solvent (AOAC 1995, Nielsen 2010), dietary fibre was evaluated using modified neutral detergent fibre method (James 1999) and available carbohydrate was measured using 3, 5-dinitrosalicylic acid (DNS) method.

#### Determination of mineral concentrations

Dried and finely powdered mushroom samples of 0.5g were taken into PTFE digestion tubes and 2ml of 65% Suprapur HNO<sub>3</sub> (Merck) and 1ml of Suprapur 30% H<sub>2</sub>O<sub>2</sub> (Merck) were added to the tubes. The samples were

### MATERIALS AND METHODS

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incinerated in CEM-MARS 5 microwave closed digestion system at 200°C. A blank digestion was carried out in a similar way without sample. For the wet digestion, temperature of the microwave system was increased up to 200°C in 27 min and kept on hold for 20 min. Digested samples were then diluted to 50ml with MiliQ water. Mineral concentrations of the mushroom samples were determined by mass spectrometer with inductively coupled plasma (ICP-MS).

#### Antimicrobial activity

##### Macro broth dilution method:

The antibacterial activity of mushrooms was evaluated using macro broth dilution method. The seven bacterial strains include two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* two Gram-positive bacteria, *Enterococcus faecalis* and *Staphylococcus aureus* and three resistant strains viz. ESBL *Klebsiella*, MRSA and VRE. A two-fold dilution series down from 256 to  $9.7 \times 10^{-4}$  µg/ml concentration was prepared at twice the desired final concentration in the sterile cation adjusted Mueller-Hinton broth. Inoculum was prepared by direct colony suspension method and diluted in required volume of broth to give final bacterial density of range  $3-7 \times 10^5$  cfu/ml. Following 16-20 hours of incubation at 37°C, MIC was defined as the lowest concentration of the extract at which no visible growth was observed. Broth was used as a negative control and gentamycin (GN) was used as reference compound. For more accurate results, the absorbance of all tubes was measure at 600nm and the average percentage inhibition of organism in all dilutions were calculated.

##### Agar well diffusion method

The method was used to measure the activity of

methanolic extracts of mushrooms of higher concentrations (10, 50 and 100 mg/ml) against seven bacterial stains. LB agar plates were prepared with 7% agar. Bacterial inoculum equivalent to McFarland 0.5 was mixed with LBA medium and poured on LBA plates. Well of 7mm diameter were perforated into the surface of the agar plates and for each test organism, the wells were filled with three different concentrations of antimicrobial solution in triplicates. Gentamycin of 2 or 5mg/ml concentration was used as reference and sterile distilled water was used as a negative control. Following incubation at 37°C, zone of inhibition (in mm) was measured.

#### Statistical analysis

All the assays were carried out in triplicate for each sample. The results are expressed as mean values and standard deviation (S.D.)

## RESULTS AND DISCUSSION

#### Proximate composition

Nutritive value of food is usually measured by the proximate composition which includes moisture, ash, protein, crude fat, crude fibre, carbohydrate content in percentage. In the present study, the proximate analysis of *Podaxis pistillaris* and *Termitomyces heimii* was measured on dry weight basis and was expressed in percentage or in g/100g (Table 1). It was found that both the mushrooms are good source of basic food composition. The nutritive value of *Podaxis pistillaris* was relatively higher than *Termitomyces heimii*, in having very high amount of protein ( $39.14 \pm 3.20\%$ ), high level of dietary fibre ( $34.33 \pm 0.58\%$ ), sufficient reduced carbohydrate ( $37.22 \pm 0.94\%$ ), low ash content ( $4.56 \pm 0.19\%$ ) and very less amount of crude fat ( $0.89 \pm 0.19\%$ ).

Table 1 Proximate composition of wild edible mushrooms (g/100g, dry weight basis<sup>a</sup>)

Samples	Percentage Composition <sup>a</sup>				
	Ash	Fat	Dietary fibre	Protein	Carbohydrate
<i>Podaxis pistillaris</i>	$4.56 \pm 0.19$	$0.89 \pm 0.19$	$34.33 \pm 0.58$	$39.14 \pm 3.20$	$37.22 \pm 0.94$
<i>Termitomyces heimii</i>	$3.26 \pm 0.05$	$4.22 \pm 0.77$	$28.33 \pm 1.53$	$18.39 \pm 1.23$	$24.46 \pm 0.76$

<sup>a</sup>Each value is expressed as mean  $\pm$  SD (n=3)

#### Ash content

The ash content in *Podaxis pistillaris* and *Termitomyces heimii* was  $4.56 \pm 0.19$  and  $3.26 \pm 0.05$  respectively. Mridu and Atri, (2017) reported higher ash content in *Calocybe gambosa* ( $10.7 \pm 0.15$ ), *Podaxis pistillaris* ( $1.36 \pm 0.24$ ) and *Lentinus squarrosulus* ( $11.4 \pm 0.55$ ) mushrooms from Haryana than the presently investigated mushrooms. Lalotra *et al.* (2018) reported higher ash content in *Amanita rubescens* ( $7 \pm 0.5$ ), *Boletus edulis* ( $6.4 \pm 0.38$ ), *Geopora arenicola* ( $9.9 \pm 0.11$ ), *Morchella deliciosa* ( $7.0 \pm 0.15$ ) and *Sparassis crispa* ( $4.9 \pm 0.11$ ) in comparison to its amount in *Podaxis pistillaris*. Similarly, Altaf *et al.* (2020) also reported comparatively higher percentage of ash in *Apioperdon pyriforme* ( $9.5 \pm 0.54$ ), *Helvella elastica* ( $10.4 \pm 1.1$ ), *Morchella conica* ( $14.7 \pm 1.08$ ) and *Rhizopogon luteolus* ( $11.0 \pm 0.88$ ).

#### Protein content

Protein content on dry weight basis in *Podaxis pistillaris* ( $39.14 \pm 3.20$ ) was found to be significantly higher than *Termitomyces heimii* ( $18.39 \pm 1.23$ ) and other mushrooms including in *Calocybe gambosa* ( $20.22 \pm 0.07$ ), *Podaxis pistillaris* ( $14.54 \pm 0.18$ ) and *Lentinus squarrosulus* ( $14.45 \pm 0.07$ ) from Haryana reported by Mridu and Atri (2017). Similarly, Lalotra *et al.* (2018) while working with mushrooms of Jammu and Kashmir reported lower amount of protein in *Amanita rubescens* ( $21.8 \pm 0.76$ ), *Boletus edulis* ( $31.3 \pm 1.15$ ), *Geopora arenicola* ( $23.8 \pm 0.76$ ), *Morchella deliciosa* ( $25.6 \pm 1.19$ ) and *Sparassis crispa* ( $16.2 \pm 0.72$ ) as compared to *Podaxis pistillaris* evaluated during the present study. Similarly, Altaf *et al.* (2020) also reported lower protein content in *Apioperdon pyriforme* ( $11.5 \pm 1.25$ ), *Helvella elastica* ( $18.0 \pm 1.45$ ), *Morchella conica* ( $24.5 \pm 1.92$ )

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and *Rhizopogon luteolus* (18.2±1.41) from Jammu and Kashmir when compared with that of *Podaxis pistillaris*.

### Fat content

Amount of fat content on dry weight basis in *Podaxis pistillaris* (0.89±0.19) was found to be significantly lower than the fat content present in *Termitomyces heimii* (4.22±0.77). When compared with the amount of fat present in *Agaricus bisporus* (2.12%), *Pleurotus florida* (1.54%), *Russula delica* (5.38%) and *Lyophyllum decastes* (2.14%) reported by Teklit (2015) from Ethiopia, its proportion was found to be much less. Similarly, Mridu and Atri (2017) also reported higher fat content in *Calocybe gambosa* (1.33±0.31) and *Podaxis pistillaris* (1.97±0.16) from Haryana but in case of *Lentinus squarrosulus* (0.33±0.44) its proportion was on the lower side.

### Fibre content

Results of present investigation showed good amount of dietary fibre in both the mushrooms. Fibre content in *Podaxis pistillaris* (34.33±0.58) and *Termitomyces heimii* (28.33±1.53) is relatively higher when compared with other mushrooms, namely, *Calocybe gambosa* (6.04±0.04) and *Podaxis pistillaris* (23.87±2.02) as reported by Mridu and Atri (2017) however, it was on the higher side in *Lentinus squarrosulus* (38.38±1.96) from Haryana. Lalotra *et al.* (2018) estimated significantly lower amount of crude fibre in *Amanita rubescens* (3.9±0.05), *Boletus edulis* (4.3±0.55), *Geopora arenicola* (5.7±0.68), *Morchella deliciosa* (5.5±0.29) and *Sparassis crispa* (6.5±0.50) in comparison to *Podaxis pistillaris* and *Termitomyces heimii*. Similarly, Altaf *et al.* (2020) also reported lower proportion of fibre content in *Apioperdon pyriforme* (4.21±0.22), *Helvella elastica* (2.6±1.21), *Morchella conica* (3.5±1.05) and *Rhizopogon luteolus* (4.82±0.51) compared to the presently evaluated *Podaxis pistillaris* and *Termitomyces heimii* from A.P.

### Carbohydrate content

The amount of reduced carbohydrate measured on dry weight basis in *Podaxis pistillaris* (37.22±0.94) and *Termitomyces heimii* (24.46±0.76) was found to be on lower side when compared with estimated values of *Agaricus bisporus* (28.38%), *Pleurotus florida* (32.08%), *Russula delica* (34.88%) and *Lyophyllum decastes* (34.36%) as reported by Teklit (2015). According to Mridu and Atri (2017) also total carbohydrate in *Calocybe gambosa* (65.61±0.73), *Podaxis pistillaris* (77.79±0.39), and *Lentinus*

*squarrosulus* (66.15±0.97) from Haryana was on the higher side in comparison with the presently investigated mushrooms from Andhra Pradesh.

### Analysis of macro elements

In the present study, macro mineral elements (Mg, K, P and Ca) concentration was measured in mg/kg on a dry weight basis (Table 2). Both the mushrooms were found to be a good source of macro nutrient elements. *Termitomyces heimii* showed high amount of potassium (9015.33±96.14) and calcium (144.14±5.90) than *Podaxis pistillaris* (8554.86±113.36 (K); 64.85±21.01 (Ca)). The higher amount of magnesium content was observed in *Termitomyces heimii* (6283.39±142.61mg/kg) and *Podaxis pistillaris* (6275.66±110.53mg/kg) when compared with the magnesium content of in *Cantharellus cibarius* (866.3±11.9) *Russula delica* (688.7±5.25), *Boletus aureus* (755.1±7.33) and *Armillaria mellea* (1063.1±7.71) from Greece reported by Ouzouni *et al.* (2009). Similarly, Altaf *et al.* (2020) while working with mushrooms of Jammu and Kashmir also reported lower amount of magnesium content in *Apioperdon pyriforme* (22.4±1.99), *Helvella elastica* (51.6±1.02), *Morchella conica* (55.5±3.1) and *Rhizopogon luteolus* (31.2±1.9) than that of *Podaxis pistillaris* and *Termitomyces heimii* evaluated presently. Potassium concentration in the presently evaluated *Termitomyces heimii* and *Podaxis pistillaris* was found to be considerably lower in comparison to the values reported by Genccelep *et al.* (2009) in *Morchella vulgaris* (20.4 mg/g), *Agaricus campestris* (18.07 mg/g) *Cantharellus cibarius* (16.5 mg/g), *Pleurotus eryngii* (17.5 mg/g) and *Pleurotus ostreatus* (21.9 mg/g) from Turkey. Similarly, even the phosphorus content evaluated by Genccelep *et al.* (2009) in *Morchella vulgaris* (2.92 mg/g), *Agaricus campestris* (2.36mg/g), *Pleurotus eryngii* (3.45mg/g) and *Pleurotus ostreatus* (3.26 mg/g) was found to be on the higher side than that observed in *Termitomyces heimii* (2050.97±41.14 mg/kg) and *Podaxis pistillaris* (2058.03±59.1 mg/kg) while in *Cantharellus cibarius* (0.64 mg/g) the phosphorus content was found to be significantly on the lower side. Calcium content in *Morchella vulgaris* (0.87 mg/g), *Agaricus campestris* (0.24mg/g) *Cantharellus cibarius* (0.9 mg/g), *Pleurotus eryngii* (0.84 mg/g) and *P. ostreatus* (1.26 mg/g) as reported by Genccelep *et al.* (2009) was found to be significantly higher in comparison to the amount of calcium evaluated in the presently studied *Termitomyces heimii* (144.14±5.9 mg/kg) and *Podaxis pistillaris* (64.85±21.01 mg/kg).

Table 2 Macro element content (mg/kg, dry weight basis<sup>a</sup>) in wild edible mushrooms

Macro element	<i>Podaxis pistillaris</i>	<i>Termitomyces heimii</i>
Magnesium (Mg)	6275.66 ± 110.53	6283.39 ± 142.61
Potassium (K)	8554.86 ± 113.36	9015.33 ± 96.14
Phosphorus (P)	2058.03 ± 59.10	2050.97 ± 41.14
Calcium (Ca)	64.85 ± 21.01	144.14 ± 05.90

<sup>a</sup>Values expressed are means ± SD

### Analysis of micro elements

Eight micronutrient elements (Mn, Fe, Cu, Zn, Co, Se, B and Mo) were determined in the presently evaluated

mushroom samples using inductively coupled plasma mass spectrometry in mg/kg unit on a dry weight basis. Data obtained from the analysis have been given in (Table 3).

Present study revealed that the amount of micronutrients in *Termitomyces heimii* was significantly higher than *Podaxis pistillaris*. In the present study, manganese (Mn) concentration in *Termitomyces heimii* ( $264.72 \pm 12.97$  mg/kg) was found to be significantly on the higher side than the

amount of manganese present in all other mushrooms as reported by Ouzouni *et al.* (2007), Genccelep *et al.* (2009), Colak *et al.* (2009), Ouzouni *et al.* (2009), Sarikurkcü *et al.* (2010), Lalotra *et al.* (2018).

Table 3 Micro element content (mg/kg, dry weight basis<sup>a</sup>) in wild edible mushrooms

Micro element	<i>Podaxis pistillaris</i>	<i>Termitomyces heimii</i>
Manganese (Mn)	$61.08 \pm 11.42$	$264.72 \pm 12.97$
Iron (Fe)	$128.04 \pm 0.89$	$501.84 \pm 08.25$
Copper (Cu)	$625.45 \pm 06.32$	$372.30 \pm 14.60$
Zinc (Zn)	$549.09 \pm 82.58$	$906.90 \pm 03.50$
Cobalt (Co)	$0.46 \pm 0.02$	$5.30 \pm 0.03$
Selenium (Se)	$27.36 \pm 0.90$	$24.02 \pm 0.82$
Boron (B)	$7.27 \pm 0.27$	$7.28 \pm 0.17$
Molybdenum (Mo)	$3.95 \pm 0.32$	$1.32 \pm 0.05$

<sup>a</sup> Values expressed are means  $\pm$  SD

The level of iron (Fe) in *Termitomyces heimii* ( $501.84 \pm 8.25$  mg/kg) was found to be relatively on the higher side in comparison to the mushrooms evaluated by Ouzouni *et al.* (2007), Ouzouni *et al.* (2009). As compared, Genccelep *et al.* (2009) in case of *Pleurotus ostreatus* (682 mg/kg) and Colak *et al.* (2009) in case of *Lycoperdon perlatum* ( $550 \pm 15.0$ ) reported higher amount of iron than both the presently evaluated mushrooms. Similarly, Altaf *et al.* (2020) also analyzed lesser iron content in mushrooms from Jammu and Kashmir except in *Morchella conica* ( $531 \pm 20.0$  ppm) and *Rhizopogon luteolus* ( $547 \pm 19.88$  ppm) where it was found higher than both the presently studied mushrooms.

The amount of copper (Cu) determined was also significantly higher in *Podaxis pistillaris* ( $625.45 \pm 6.32$  mg/kg) when compared with other mushrooms evaluated by Ouzouni *et al.* (2007), Genccelep *et al.* (2009), Colak *et al.* (2009), Ouzouni *et al.* (2009), Sarikurkcü *et al.* (2010), Sarikurkcü *et al.* (2015), Lalotra *et al.* (2018), Altaf *et al.* (2020) in this regard. Similarly zinc content measured in *Termitomyces heimii* ( $906.9 \pm 3.5$  mg/kg) was found to be considerably on higher side as documented by Ouzouni *et*

*al.* (2007), Genccelep *et al.* (2009), Colak *et al.* (2009), Ouzouni *et al.* (2009), Sarikurkcü *et al.* (2010), Sarikurkcü *et al.* (2015), Lalotra *et al.* (2018), Altaf *et al.* (2020) in different mushrooms evaluated by them from time to time.

Study revealed that cobalt (Co) content in *Termitomyces heimii* ( $5.30 \pm 0.03$  mg/kg) was also relatively on the higher side in comparison to the value of this micronutrient obtained by Ouzouni *et al.* (2007), Sarikurkcü *et al.* (2015) while working with some of the mushrooms except for *Lycoperdon perlatum* ( $13.9 \pm 0.03$  mg/kg) in which the documented amount of cobalt was much more in comparison.

In the present study, the detected amount of selenium (Se) and molybdenum (Mo) in *Podaxis pistillaris* (Se- $27.36 \pm 0.9$  mg/kg; Mo- $3.95 \pm 0.32$  mg/kg) was more in comparison to their values documented in *Termitomyces heimii* (Se- $24.02 \pm 0.82$  mg/kg; Mo- $1.32 \pm 0.05$  mg/kg) whereas the amount of boron (B) was found to be almost equal in *Podaxis pistillaris* ( $7.27 \pm 0.27$  mg/kg) and *Termitomyces heimii* ( $7.28 \pm 0.17$  mg/kg) during the present investigations.

Table 4 Antimicrobial activity of methanolic extracts of mushrooms using broth dilution method<sup>a</sup>

Bacteria	MIC ( $\mu$ g/ml) <sup>b</sup>		
	<i>Podaxis pistillaris</i>	<i>Termitomyces heimii</i>	Gentamycin
<i>Escherichia coli</i>	0.5(++) (36.08%)	0.25 (+) (1.78%)	2(+++++)
<i>Pseudomonas aeruginosa</i>	256(+) (0.12%)	128 (++) (29.54%)	1 (+++++)
<i>Staphylococcus aureus</i>	(-)	0.25(++) (32.54%)	0.25(+++++)
MRSA	1(+) (12.05%)	0.03125(+) (3.74%)	4(+) (19.93%)
ESBL <i>Klebsiella</i>	0.03125(++) (34.97%)	0.0019 (+) (19.21%)	16(+++++)
<i>Enterococcus faecalis</i>	1(+++) (52.26%)	0.5(+) (19.53%)	4(+++++)
VRE	0.5(+) (9.46%)	32(++) (36.50%)	2(+) (17.03%)

<sup>a</sup> In macrobroth dilution method, 20 dilutions were used ( $256$  to  $9.7 \times 10^{-4}$   $\mu$ g/ml) and average inhibition percentage is given (n=3)

<sup>b</sup> Antibacterial activity is based on inhibition percentage: very low activity (+,  $\leq 20\%$ ), low activity (++,  $>20\%$  and  $\leq 40\%$ ), moderate activity (+++,  $>40\%$  and  $\leq 60\%$ ), high activity (++++,  $>60\%$  and  $\leq 90\%$ ) and MIC (++++,  $> 90\%$ ). No antibacterial activity “-”

#### Antimicrobial activity

Macrobroth dilution method and agar-well diffusion method were used to determine the antimicrobial activity of *Podaxis pistillaris* and *Termitomyces heimii*. MICs values

for each bacterium were measured based on the average inhibition percentage and extracts of *Podaxis pistillaris* were found to be more effective with moderate antibacterial activity than *Termitomyces heimii* showing low antibacterial

activity (Table 4). Similarly, antimicrobial activities in terms of zone of inhibition for mushroom extracts at higher concentrations were tested against same set of bacteria using Agar-well diffusion method (Table 5). The standard reference antibiotic, Gentamycin sulfate (GN) was found to be effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *ESBL Klebsiella* and

*Enterococcus faecalis* with a MIC at 2µg/ml, 1µg/ml, 0.25µg/ml, 16µg/ml, and 4µg/ml respectively (Table 4). Similar results of GN were seen in agar-well diffusion method (Table 5) in terms of zone of inhibition against *Escherichia coli* (25mm), *Pseudomonas aeruginosa* (14mm), *Staphylococcus aureus* (24mm), *ESBL Klebsiella* (18mm) and *Enterococcus faecalis* (15mm).

Table 5 Antimicrobial activity of methanolic extracts of mushrooms using agar-well diffusion method<sup>a</sup>

Bacteria	Inhibition zone (mm)						GN	Water
	<i>Podaxis pistillaris</i>			<i>Termitomyces heimii</i>				
Normal strain	100	50	10	100	50	10	2	0
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml
<i>Escherichia coli</i>	(-)	(-)	(-)	(-)	(-)	(-)	(++++)	25
<i>Pseudomonas aeruginosa</i>	(-)	(-)	(-)	(-)	(-)	(-)	(++++)	14
<i>Staphylococcus aureus</i>	(-)	(-)	(-)	(-)	(-)	(-)	(++++)	24
<i>Enterococcus faecalis</i>	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Resistant strain	100	50	10	100	50	10	5	0
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml
<i>Esbl Klebsiella</i>	(-)	(-)	(-)	(-)	(-)	(-)	(++++)	18
<i>MRSA</i>	(-)	(-)	(-)	(-)	(-)	(-)	(++++)	15
<i>VRE</i>	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

<sup>a</sup>No antibacterial activity (-), inhibition zone <1mm; low activity (+), inhibition zone 2-3 mm; moderate activity (++) , inhibition zone 4-5 mm; high activity (+++), inhibition zone 6-9 mm; strong activity (++++), inhibition zone > 9 mm

Methanolic extract of *Podaxis pistillaris* showed low antimicrobial activity (36.08% inhibition) against *Escherichia coli* at 0.5µg/ml dilution whereas with the extracts of *Termitomyces heimii* the percentage inhibition was found to be very low activity (1.78% inhibition) at 0.25µg/ml. The results obtained presently are in conformity with some of the previous studies where antimicrobial activities in terms of MIC values and zone of inhibition for *E. coli* were found to be resistant to various extracts of mushrooms (Turkoglu *et al.* (2007), Kitzberger *et al.* 2007, Barros *et al.* 2007, Barros *et al.* 2008, Ozturk *et al.* 2011, Ren *et al.* 2014). There are very few reports where methanolic extracts of mushrooms have shown antimicrobial activity against *E. coli* in terms of MIC by Alves *et al.* (2012) and by Ahmad *et al.* (2012) in terms of zone of inhibition.

Very low antibacterial activity was observed in *Podaxis pistillaris* at 256µg/ml and low activity (29.54% inhibition) in *Termitomyces heimii* at 128µg/ml for *Pseudomonas aeruginosa* in the present study which was in concordance with previous literature where *Pseudomonas aeruginosa* were found to be resistant to various extracts of mushrooms reported by Turkoglu *et al.* (2007), Barros *et al.* (2007) except in *Lactarius deliciosus* (100mg/ml); Barros *et al.* (2008), Ozturk *et al.* (2011). But there are reports where activities were evaluated for *Pseudomonas aeruginosa* in terms of zone of inhibition by Ahmad *et al.* (2012) and in terms of MIC values by Nowacka *et al.* (2014).

In the present study no antimicrobial activity was reported against *Staphylococcus aureus* from the various dilutions of *Podaxis pistillaris* which was in accordance with the activities for *Staphylococcus aureus* found to be resistant to various extracts of mushrooms reported by Solak *et al.* (2006), Turkoglu *et al.* (2007), Kitzberger *et al.*

(2007), Ozturk *et al.* (2011) except in *Agaricus bitorquis* (12mm). But *Termitomyces heimii* showed moderate activity (32.54% inhibition) for *Staphylococcus aureus* at 0.25µg/ml. Similarly, activities were reported in terms of zone of inhibition (in mm) for *Staphylococcus aureus* in *Pleurotus ostreatus* (11±0.17mm), *Pleurotus sajor-kaju* (10.75±0.75mm) and *Morchella esculenta* (14±0.36mm) by Ahmad *et al.* (2012) whereas in terms of MIC values in *Armillaria mellea* (2.5mg/ml), *Clitocybe gibba* (2.5mg/ml), *Lycoperdon perlatum* (2.5mg/ml) and *Macrolepiota procera* (2.5mg/ml) by Nowacka *et al.* (2014).

*Podaxis pistillaris* showed moderate activity (52.26% inhibition) against *Enterococcus faecalis* at 1µg/ml dilution of methanolic extract whereas *Termitomyces heimii* showed very low activity (19.53% inhibition) at 0.5µg/ml dilution. Similar activity was reported by Ren *et al.* (2014) where MIC values for *Enterococcus faecalis* were found to be resistant to polysaccharide extracts of all mushrooms. Both the mushrooms and Gentamycin showed very low antimicrobial activity against *Methicillin-Resistant Staphylococcus aureus* (MRSA) whereas Alves *et al.* (2012) reported higher MIC values in *Agaricus arvensis* (>20mg/ml), *Agaricus bisporus* (>20mg/ml), *Cantharellus cibarius* (>20mg/ml), *Lactarius deliciosus* (>20mg/ml) and *Russula delica* (10mg/ml) against MRSA.

Methanolic extract of *Podaxis pistillaris* has shown 34.97% inhibition (low activity) of *Extended-Spectrum β-Lactamase Klebsiella* (resistant strain) at 1µg/ml dilution whereas *Termitomyces heimii* has shown 19.21% inhibition at 0.0019µg/ml. Similarly, extracts of *Termitomyces heimii* revealed 36.50% *Vancomycin Resistant Enterococci* (VRE) inhibition at 32µg/ml dilution while *Podaxis pistillaris* showed 9.46% inhibition (very low activity) at 0.5µg/ml. There is no antibacterial activity report of mushrooms

against ESBL *Klebsiella* and VRE from the previous literature.

Although the antimicrobial activity investigated in the present study for both the mushrooms is in the range of very low to moderate than the reference antibiotic, it confirms that the extracts of mushrooms may have antibiotic molecules. Since these extracts are not a purified form of antimicrobial agent, the activity expressed is low.

The results of the current study are the first information on antimicrobial activity and mineral elements profile of *Podaxis pistillaris* and *Termitomyces heimii* from Andhra Pradesh of India. The proximate analysis revealed that these wild edible mushrooms are highly nutritive due to their high content of protein, good amount of dietary fibre, sufficient carbohydrate and low level of fat. Both the mushrooms are also good source of macro and micro nutrient elements. *Termitomyces heimii* evaluated with high amount of Mg, K and Ca but the level of phosphorus was reported to be higher in *Podaxis pistillaris*. In addition to this, significant

concentrations of manganese iron, zinc and cobalt were evaluated in *Termitomyces heimii* and *Podaxis pistillaris* reported higher levels of copper, selenium and molybdenum. Boron contents were found relatively equal in both the mushrooms. Furthermore, the methanolic extracts of the mushrooms at higher dilutions showed very low to moderate antimicrobial activity against normal and their resistant bacterial strains whereas concentrated extracts being crude mixture of compounds were not effective against any test organism.

### Conflict of interest

The authors declare that there are no conflicts of interest.

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