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IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF EDIBLE MUSHROOM (*TERMITOMYCES HEIMII)*

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ABSTRACT

Termitomyces heimii popularly known as "Puttakokulu" in tribal area of Visakhapatnam. This edible wild mushroom was consumed by communities living in a tribal area of Visakhapatnam, Andhra Pradesh, India. The aim of the present study is to determination of antioxidant and antimicrobial activity of hexane, methanol, chloroform and distilled water extracts of *T. heimii* was tested. The antioxidant activities of was evaluated by using three different methods that are Reducing power, FRAP and DPPH were analysed. The methanolic extract of *T. heimii* gave a higher reducing power and antioxidant activity was present. Similarly, methanolic extract of *T .heimii* gives excellent antimicrobial activity against bacterial pathogens.

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INTRODUCTION

Edible mushrooms are widely consumed in many countries and the amount consumes has greatly increased because of their good taste, ease of purchase and attraction as functional foods since they are low in calories, sodium, fat and cholesterol while high in protein, carbohydrate, fibre, vitamins and the important content of essential amino acids (Mattial *et al.*,2000). Wild edible mushrooms (WEMs) are important contributions to rural and tribal livelihoods (Christenses *et al*. 2008). For many years various macro fungal species have been used worldwide in preparing dishes with high protein and mineral content. Despite this, WEMs are seldom included in valuation of tropical forests, which has traditionally been mostly based on a financial appraisal of timber stock. Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immune stimulatory effects, have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities (Oyetayo *et al.,* 2009; Mau *et al.,* 2004; Barros *et al.,* 2007; Ferreira *et al*., 2007).

Termitophilic fungi are a monophyletic group of gilled mushrooms belonging to the genus *Termitomyces*. Aanen & Eggleton (2006) revealed that cultivation of *Termitomyces* by termites originated in the African rain forests as the main centre and migrated to other geographical regions like Asia and Madagascar. In the tribal area of Visakhapatnam *Termitomyces spices* are commonly observed in my study they have been present at rainy seasons. The tribal people they have been consumed as a food. So the aim of the present study to determining the antioxidant activity, the reduction power, the inhibition of free radicals, and the antimicrobial activity of *Termitomyces heimii.*

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MATERIAL AND METHODS

Study Area

Paderu Division of Visakhapatnam District, Andhra Pradesh, is the higher altitude zone in the hilly tracts of Eastern Ghats of Andhra Pradesh. It has the second highest tribal population in Andhra Pradesh. It lies in between latitudes of 18.0833^o north and longitude of 82.667^o East with a total geographical area of 3, 24,965 Ha. Out of which the forest area under the control of the Division is 104811.91 Ha. The division comprises of a series of hills having an altitude ranging from 900 to 1680 mtrs above M.S.L. The area receives an average annual rainfall of 1800 mm and support a rich diversity of plant wealth. It includes three Forest Ranges i.e. 1.Araku Forest Range 2.Paderu Forest Range and 3.Pedabayalu Forest Range (Padal et al., 2010).

Collection Samples

Termitomyces heimii, samples were collected from different field sites in Paderu Mandal (Figure 1) during the period of 2014 to 2016. They were then identified using colored field guide books, monographs (Kirk *et al.,* 2001; Lodge *et al.,* 2004; De greef, 2010) and internet facility shown in Fig:2. After freshly observation mushroom were sundried and deposited at the Andhra University and a duplicate of *T. heimii* is also available at herbarium of Botany department.

Preparation of extracts

Soxhlet extraction

The dried and powdered materials (20 gm) were extracted with 200 ml of each solvent separately by using soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent. The solvents used for the study were Methanol. The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4º C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml. The dry extracts were stored at -18°C until used in the tests. The extracts were dissolved in 5% dimethyl sulphoxide (DMSO).

Antioxidant activity

DPPH radical scavenging activity method

The scavenging activity of mushrooms was estimated according to the procedure described by Shimada *et al.* (1992) with some modifications. Firstly, an aliquot of 0.5 ml of sample extracts at different concentrations were added to test tubes with 2.9 ml of 200 µM DPPH radical in ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-vis spectrophotometer. Extraction solvent was used as blank while mixture without extract served as control. Ascorbic acid was used as a standard. The scavenging effect was calculated based on the following equation:

Scavenging effect (%) = 1- [(Absorbance sample/ Absorbance control) x 100]

EC50 value (mg/ml) was defined as the total antioxidant needed to decrease the initial DPPH free radicals by 50%. It was determined from the plotted graph of scavenging activity against various concentrations of the sample extracts.

Reducing power

The reducing power of extracts was determined by the method of Oyaizu (1986). 1 ml of extracts were mixed with 2.5 ml of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated at 50^{o}C for 20 min. After that, trichloroacetic acid (10%, 2.5 ml) was added to the mixture and centrifuged. Finally, the upper layer was mixed with distilled water (2.5ml) and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance of the solution was measured at 700 nm in spectrophotometer.
Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid and BHA were used as positive control.

Antibacterial activity

Test of Microorganisms

Pure culture of *Staphylococcus aureus (MTCC-3160), Streptococcus pyogenes (MTCC-2327), Klebsiella pneumonia (MTCC-452) and Escherichia coil (MTCC-443),* were obtained from department of Microbiology laboratory, AU and was sub-cultured on nutrient agar to ensure the purity of the culture and the pure isolate identified and used for present study.

Agar well diffusion method

The antimicrobial assay was performed by agar well diffusion method (Perez *et al.,* 1990) for solvent extract. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 100µl of the inoculums (1x 10^8Cfu) and poured into the sterile petri plates. In agar well diffusion method, a well was prepared in the plates with help of cork-borer (8mm). 50 to 400µl of test compound were introduced into well. The plates were incubated overnight at 37^{o}C.

RESULTS AND DISCUSSION

The methanol extract of mycelia was subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method. Scavenging the stable DPPH radical is widely used method to evaluate the antioxidant activity in comparison to other method because this method is simple, requires short period of time and sensitive. DPPH is a stable free radical that shows a characteristic absorbance at 517 nm, which decreases significantly when exposed to radical scavengers by providing hydrogen atom or electron to be a stable diamagnetic molecule. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Scavenging effects of methanol extracts from mushrooms on DPPH radical increased with the increased concentrations. According to the Table1, ***T. heimii*** showed higher radical scavenging activity which was 87.32% in 400 µl / ml concentration. This study suggests that the antioxidant activity of methanolic extracts from mushrooms is due to phenolic compounds content. In research conducted by Aneta Slawinska et al. (2013) was reported. The antioxidant activity by neutralizing the linoleate free radicals and other free radicals formed in the system exhibited by *Termitomyces heimii* and their EC50 value was around 2.63 mg/mL in the extracts.

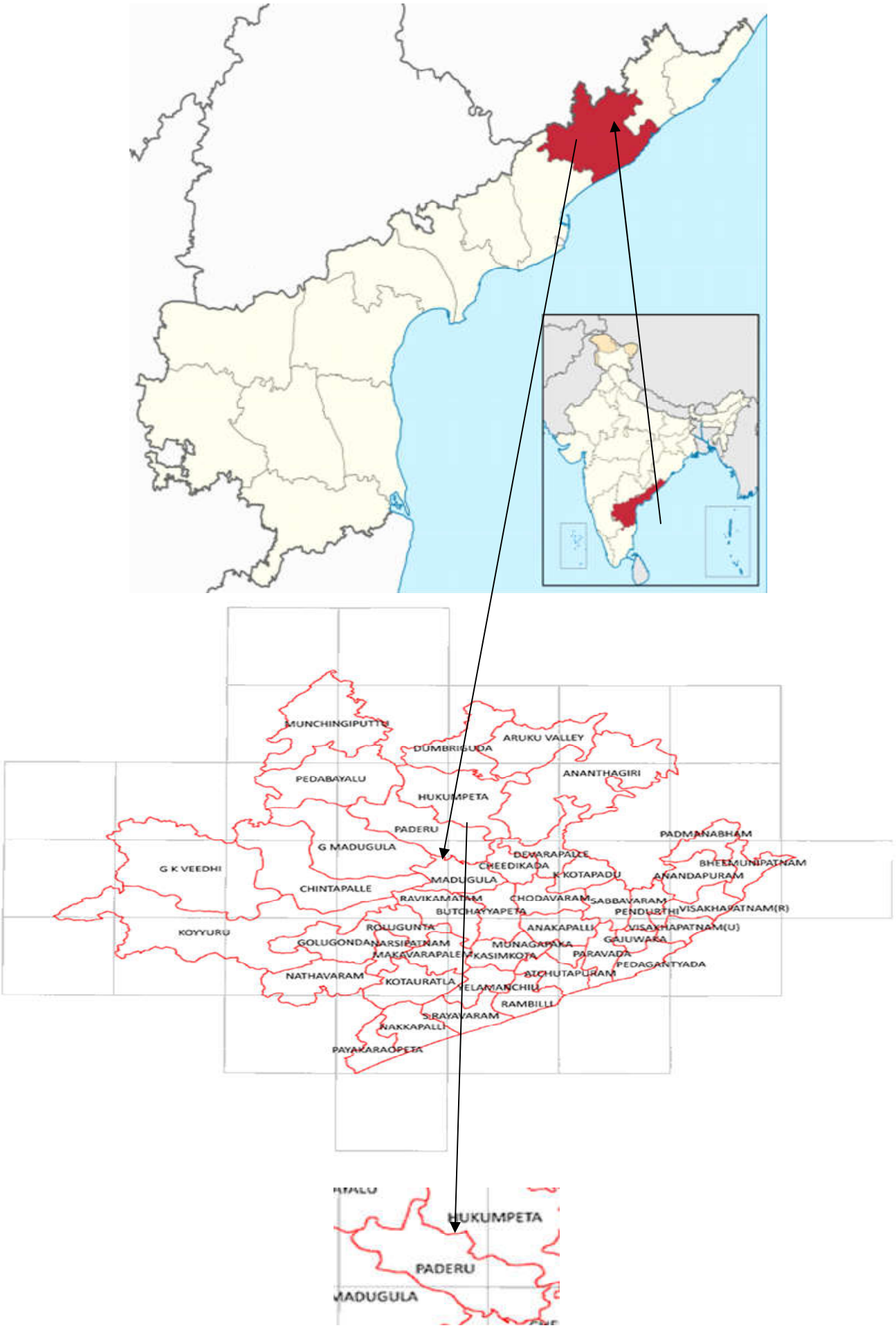

Fig 1. Study Area Paderu

Fig. 2. Different stages of *Termitomycesheimii* mushroom grown in Coffee agroforests soil

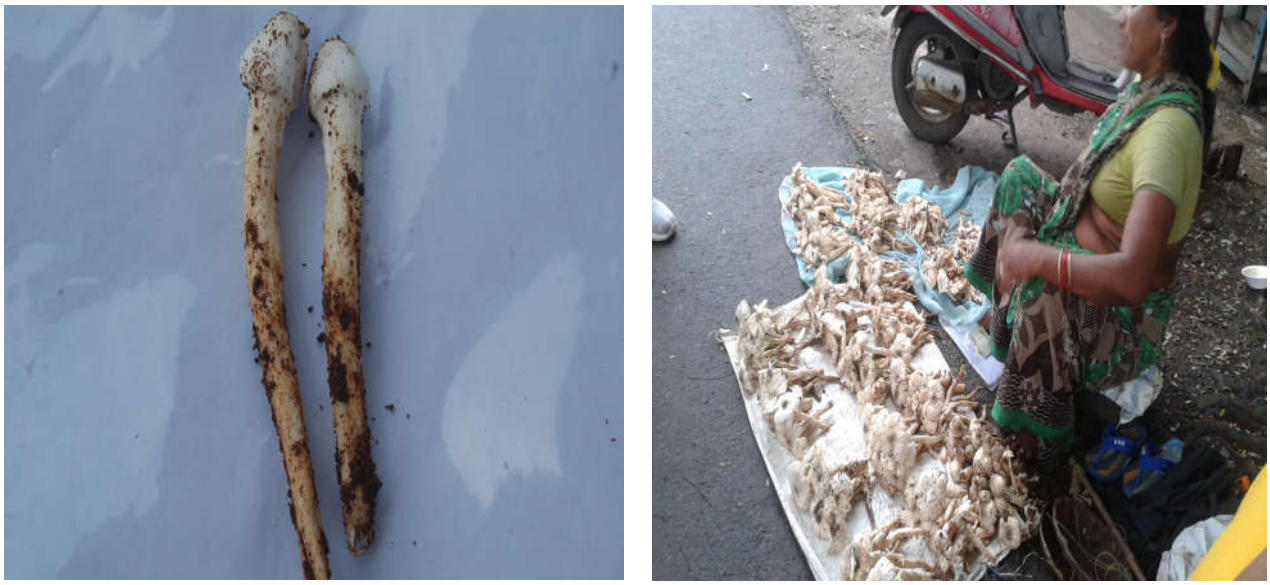

Table 1. Scavenging Effects of *T.heimii*

	50µl	100µl	150µl	200µl	250µl	300µl	400µl
DPPH	21.75%	53.59%	55.82%	68.88%	75.54%	76.48%	87.32%
Reducing power	0.0236	0.0462	0.0952	0.1019	0.1144	0.1504	0.2418

Table 2. Antimicrobial Activity of *T.heimii*

Test Microorganisms	50(µg/ml)	100(µg/ml)	150(µg/ml)	200(µg/ml)
Staphylococcus aureus	10	11	12	15
Streptococcus pyogenes	13	14	16	18
Klebsiella pneumoniae	15	15	18	20
Escherichia coli	12	15	13	22
Pseudomonas	10	12	14	18

Similar results were reported from the edible mushrooms like *L. gigantus, S. imbricatus and Agaricus aruensis*. Reducing powers of methanolic extracts from mushrooms mycelia were excellent and increased steadily increased with lower to higher concentrations (50 - 200µl/ml), which were shown in Graph 2. The highest reducing powers were found in. The difference scavenging effect among solvent extraction was due to that free radical scavenging activity is species-dependent. Besides, most the scavenging activities were probably due to light sensitivity of the DPPH radical although with variations of extracts (Masalu *et al.,* 2012). In the present study methanol extract of Termitomyces heimii showed highest zone of inhabitation present against *E.coli* that is 22 mm at 200 µg/ml concentration, lowest zone of inhibition 10mm observed both *S.aureus* and *Pseudomonas* shown in fig3. These results confirm that bioactive components of mushroom may differ in their solubility depending on the extractive solvents. Antimicrobial activity in natural source extracts depends not only on the presence of phenolic compounds but also on the presence of various secondary metabolites.

Conclusion

The results of this study confirm the potential of macromycetes as an antimicrobial and an antioxidant agent. *Termitomyces heimii* present a valuable source of antimicrobial and antioxidant agents, but require further studies to optimize the growth conditions and extraction methods to recover larger amounts of biologically active compounds. Potential therapeutic implications of mushrooms are enormous, but detailed mechanisms of the various health benefits still require investigation. Exploring antioxidant and antimicrobial activity of mushrooms and isolation of active compounds with therapeutic value remains a challenge and still much work is to be done in this area of research. In conclusion it can be stated that tested *Termitomyces heimii*

extract have a strong antioxidant and antimicrobial activity in vitro. Based on these results, *Termitomyces heimii* appear to be good could be of significance in human therapy, animal and plant diseases further studies should be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant and antimicrobial activity.

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