Antioxidant Activity of Wild Mushrooms of Chhattisgarh with Special Reference to *Termitomyces heimii*

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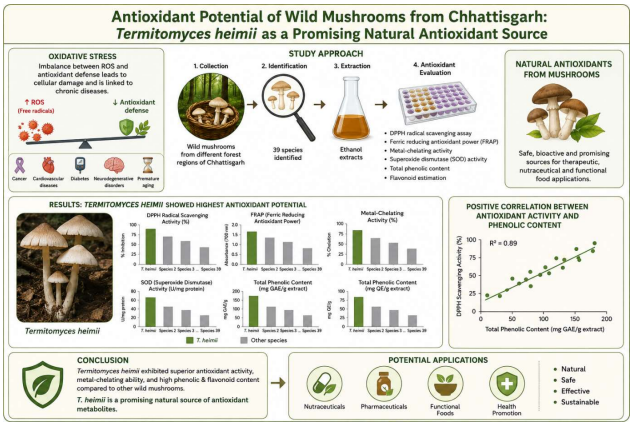
Abstract



Living cells, including those of humans, animals, and plants, are perpetually subjected to several stressors that induce oxidative stress. Oxidative stress occurs in a biological system due to heightened exposure to oxidants, diminished antioxidant capacity, or a combination of both factors. It is frequently linked to or results in the production of reactive oxygen species (ROS), including free radicals. The excessive generation of reactive oxygen species (ROS) and free radicals implicated in the pathogenesis of several chronic disorders including cancer, cardiovascular diseases, diabetes, neurodegenerative disorders, and premature aging. Natural antioxidants derived from edible and medicinal mushrooms have attracted considerable attention owing to their safety and therapeutic potential. The present study aimed to evaluate the antioxidant activity of wild mushrooms collected from different forest regions of Chhattisgarh with special reference to *Termitomycesheimii*. A total of 39 mushroom species belonging to different taxonomic groups were collected, identified, and screened for antioxidant activity. Ethanol extracts were subjected to DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP), metal-chelating activity, superoxide dismutase (SOD) activity, total phenolic content, and flavonoid estimation. Among all investigated species, *T. heimii* exhibited the highest antioxidant potential with remarkable free radical scavenging activity, metal-chelating ability, and elevated concentrations of phenolic and flavonoid compounds. The results demonstrated a positive correlation between antioxidant activity and phenolic content. The findings suggest that *T. heimii* represents a promising natural source of antioxidant metabolites and may be explored for nutraceutical, pharmaceutical, and functional food applications.

Keywords: Wild mushrooms, *Termitomyces heimii*, antioxidant activity, DPPH, Phenolics, flavonoids,

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1. Introduction

Reactive oxygen species (ROS) generated during normal cellular metabolism play important roles in physiological processes; however, excessive ROS production leads to oxidative stress, causing damage to proteins, lipids, carbohydrates, and nucleic acids.[1] Oxidative stress has been associated with numerous pathological conditions including atherosclerosis, diabetes, cancer, cardiovascular diseases, Alzheimer's disease, Parkinson's disease, and aging.[2]

Antioxidants act as protective agents by scavenging free radicals, inhibiting oxidative chain reactions, and maintaining cellular redox balance. Due to concerns regarding the toxicity of synthetic antioxidants such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), considerable interest has shifted toward natural antioxidant sources.[3]Mushrooms have emerged as important functional foods because of their nutritional composition and diverse bioactive metabolites. They are rich sources of proteins,

dietary fibers, vitamins, minerals, polysaccharides, phenolic compounds, flavonoids, terpenoids, and other secondary metabolites possessing antioxidant properties[4]the genus Termitomyces, which is widely distributed in tropical regions, has gained attention for its antioxidant properties. Researchers reported that various species of Termitomyces exhibit strong antioxidant activity when extracted using solvents such as methanol and ethanol. The study also highlighted that polysaccharides extracted from Termitomyces species effectively reduce ROS levels and enhance antioxidant defense systems.[5,6]

India harbors rich fungal biodiversity, and the forests of Chhattisgarh provide favorable ecological conditions for the growth of numerous edible and medicinal mushroom species. Although several mushrooms are traditionally consumed by tribal communities, their antioxidant potential remains insufficiently explored. Therefore, the present investigation was undertaken to evaluate the antioxidant activity of wild mushrooms of Chhattisgarh and identify species with superior antioxidant properties, with particular emphasis on *Termitomyces heimii*.

2. Materials and Methods

2.1 Study Area and Survey of Mushroom Diversity

The present investigation was conducted in different forest ecosystems of Chhattisgarh, India, including Bastar, Kanker, Kondagaon, Korba, Surguja, Kabirdham, Mahasamund, Raigarh, and adjoining forest regions. These areas are characterized by tropical deciduous vegetation dominated by *Shorearobusta* (Sal), *Tectonagrandis* (Teak), and mixed forest species that provide suitable ecological conditions for mushroom growth. Extensive field surveys were carried out during the monsoon seasons from 2022 to 2024, when mushroom fructification is at its peak. The objective of the survey was to document wild mushroom diversity and identify species possessing significant antioxidant activity.

2.2 Collection and Identification of Mushroom Samples

Fresh fruiting bodies of mushrooms were collected from forest floors, termite mounds, decaying logs, and leaf litter habitats. Morphological characteristics such as cap shape, pileus colour, stipe structure, gill arrangement, spore print colour, and habitat were recorded in the field. Each specimen was photographed in situ before collection.[6,7]

Samples were transported to the laboratory in sterile polyethylene bags and cleaned carefully to remove soil and debris. Taxonomic identification was carried out using standard mushroom identification manuals and mycological keys based on macro- and microscopic characteristics. Voucher specimens were preserved for future reference.

2.3 Drying and Preparation of Mushroom Powder

Collected mushroom samples were washed thoroughly with distilled water and shade-dried at room temperature for 7–10 days until constant weight was achieved. The dried fruiting bodies were pulverized using an electric grinder to obtain a fine powder. The powdered material was stored in airtight sterile containers at 4°C until extraction.

2.4 Extraction of Antioxidant Metabolites

Antioxidant compounds were extracted using Soxhlet extraction. Approximately 25g of mushroom powder was packed into a cellulose extraction thimble and extracted with ethanol for 6 hours. Ethanol was selected due to its efficiency in extracting phenolics, flavonoids, and other antioxidant metabolites.[7]

The solvent extract was concentrated under reduced pressure using a rotary vacuum evaporator at 40–45°C. The concentrated extract was further dried and stored at 4°C for antioxidant assays.

2.5 Screening of Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

The antioxidant activity was initially evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assaydescribed by Blois[8]. Different concentrations of mushroom extracts were mixed with DPPH solution and incubated in darkness for 30 minutes. The decrease in absorbance was measured spectrophotometrically at 517 nm.A lower absorbance of the reaction mixture indicated a higher free radical scavenging activity.The standard antioxidants (20µg/ml) butylatedhydroxyanisole (BHA) was used as positivecontrol.

Percentage inhibition was calculated as:

DPPH scavenging effect (%) = [(A_{DPPH} - A_{Extr})/A_{DPPH}] x 100

Where,

- $A_{DPPH}$is the absorbance value of the DPPH blank (without sample)
- $A_{Extr}$is the absorbance value of the test solution (mushroom species extract).

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of mushroom extracts was determined using the FRAP method. The extract was mixed with phosphate buffer and potassium ferricyanide and incubated at 50°C. After reaction completion, ferric chloride solution was added and absorbance was recorded at 700 nm. Elevatedabsorbance indicated greater reducing power.The reducing capacity of the ethanolic extract wasevaluated against L-ascorbic acid as the standard antioxidant.[9]

2.5.3 Metal Chelating Activity

Ferrozine may quantitatively bind with Fe2+ to generate a red-colored complex. This reaction is constrained by the presence of additional chelating agents, leading to a reduction in the red hue of the ferrozine-Fe2+ complexes. The chelating efficacy of the ethanolic extracts on Fe2+ was assessed using the method established by Decker and Wetch[10]the metal-chelating ability of extracts was evaluated using ferrous chloride and ferrozine reagent. Chelation of ferrous ions reduces formation of the ferrozine–Fe^{2+} complex. Absorbance was measured at 562 nm.

Chelating activity (%) = [(Ao-(A – Ab)/Ao] x 100

Where,

- Aois the absorbance value of the solution but without sample (control);
- A is the absorbance of sample (mushrooms extract)
- Abis the absorbance value of the sample without ferrozine (blank)

2.5.4 Superoxide Dismutase (SOD) Activity

The superoxide anion scavenging activity (SAS) of the extracts was assessed using the technique outlined by Nishimiki*et al*.[11]SOD activity was determined spectrophotometrically by measuring inhibition of superoxide radical formation. The assay evaluated the capacity of mushroom metabolites to neutralize superoxide radicals generated in the reaction system.Reduced absorbance signified enhanced superoxide anion scavenging ability. Identical quantities of the typical antioxidants butylatedhydroxytoluene (BHT) were employed as positive controls. The inhibition percentage was calculated by comparing the findings of control and test samples.

2.6 Determination of Antioxidant Compounds

Total Phenolic Content

Total soluble phenolics compounds in the mushroom ethanolic extracts were determined with FolinCiocalteu reagent according to the method of Slinkard. Total phenolic content was estimated using Folin–Ciocalteu reagent. The absorbance was measure at 760 nm. The concentration of total phenolic compounds in the mushroom ethanolic extracts determine as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graphs is given as:

Absorbance = 0.00246 pyrocatechol µg + 0.00325 (R2: 0.9996)

Total Flavonoid Content

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for freeradical generation. Flavonoid concentration was determined by the method of Kim *et al*.[12]Flavonoid content was determined using aluminum chloride colorimetric assay. Quercetin served as standard and results were expressed as µgquercetin equivalents per mg extract.[12,13]

2.7. Determination of Lipid Peroxidation:

The inhibition of lipid peroxidation (ILP) of Mushroom extracts was determined according to the method of Oyetayo.[14] An aqueous solution of linoleic acid and 2, 2'azobis, 2-amidineopropane dihydrochloride (AAPH) solution were prepared as described by Liguori*et al.*[15]

3. Results and Discussion

3.1 Survey and Collection of Mushroom Samples

Field exploration resulted in the collection of 39 mushroom species representing several taxonomic groups distributed throughout the forests of Chhattisgarh. The diversity reflected the rich fungal wealth of the region. The majority of species belonged to the families Lyophyllaceae, Agaricaceae, Ganodermataceae, Russulaceae, and Polyporaceae.

Several edible and medicinal mushrooms including *Termitomycesheimii*, *Ganodermalucidum*, *Pleurotusostreatus*, *Pisolithustinctorius*, and *Lentinuscladopus* were identified during the survey.

Table 1. Mushroom Diversity Recorded from Chhattisgarh

S.N.	Category	Number of Species
1	Edible Mushrooms	18
2	Medicinal Mushrooms	12
3	Ectomycorrhizal Mushrooms	5
4	Wood Rot Fungi	4

3.2 Screening of Mushrooms for Antioxidant Activity

All collected mushroom extracts were screened using DPPH, FRAP, metal-chelating, SOD, phenolic, and flavonoid assays. Significant variation in antioxidant potential was observed among species. The most prominent species of mushrooms with good antioxidant activity and compound were reported in table 2.

Table 2. Comparative Antioxidant Activity of Selected Mushrooms

S.N.	Species	DPPH (%)	Chelating(%)	Phenolics (µg/mg)	Flavonoids (µg/mg)
1.	*Termitomycesheimii*	90.52 ±0.09	91.55± 0.15	46.00 ±0.05	33.56 ±0.02
2.	*Ganoder*	86.65	86.78±	41.00	29.42

	malucidum	±0.02	0.11	±0.05	±0.06
3.	*Pisolithus tinctorius*	80.90 ±0.07	76.00± 0.09	34.00 ±0.07	25.15 ±0.16
4.	*Lentinuscladopus*	70.57 ±0.12	69.00± 0.04	28.00 ±0.10	18.40 ±0.03

- Data are multiple of three observations
- Values±SEM

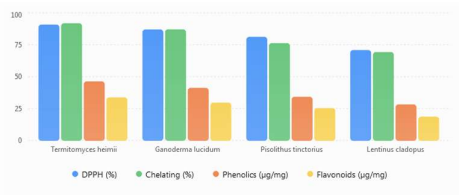


Fig.1: Comparison of Antioxidant Activity of Mushroom Species

3.3 DPPH Radical Scavenging Activity

The radical scavenging activity augmented with a higher
proportion of free radical inhibition. Potential isolates of several mushroom species were evaluated for their capacity to generate bioactive compounds by the DPPH test. The findings suggests that among all tested mushroom samples, *T. heimii*and *G. lucidum*species decrease the radical to the equivalent hydrazine by 90.52% and 86.65%, respectively indicating exceptional hydrogen-donating capacity and free radical neutralization potential.

The strong DPPH activity may be attributed to the abundance of phenolic compounds and flavonoids present in the extract. Similar findings have been reported for medicinal mushrooms such as *Ganodermalucidum* and *Pleurotus* species by Oyetayo.[16]

3.4 Metal Chelating Activity

Metal ions such as Fe^{2+} promote generation of hydroxyl radicals through Fenton reactions. Chelation of these ions is an important antioxidant mechanism.

T. heimii demonstrated the highest metal-chelating activity (91.55%), suggesting a strong ability to prevent metal-induced oxidative damage which highlighting theirpotential as antioxidants linked to this iron-binding ability. The findingssomewhat concur with those reported by Chandan*et al.*[16] and Sarithaet *al.*[17].

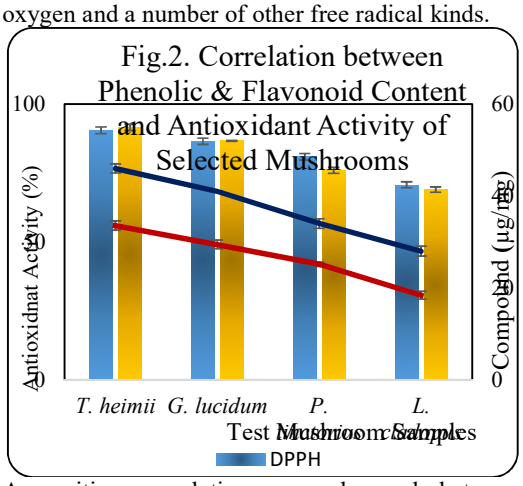
3.5 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay revealed substantial reducing power in all mushroom extracts. The reducing capacity was highest in *T. heimii*, followed by *G. lucidum*. The elevated FRAP value indicates the presence of reductones capable of donating electrons to stabilize free radicals.

3.6 Total Phenolic and Flavonoid Contents

Phenolic compounds are major contributors to antioxidant activity because of their redox properties and hydrogen-donating ability.

The total phenolic content of *T. heimii* was 46 µg mg^{-1}, the positive correlations were found between total phenoliccontent in the mushroom extracts and their antioxidant activities. The phenoliccompounds may contribute directly to antioxidative action.Various studies on total phenolic content had been published in several papers.[17,18] While flavonoid content reached 33.56 µg mg^{-1}. These values were significantly higher than those observed in other mushroomsand this flavonoidcontent increased as the concentration of the extract increased. According to Zahra[19] *et al.* (2024) research, flavonoids have been demonstrated to be highly effective scavengers of a wide variety of oxidizing chemicals, including singlet oxygen and a number of other free radical kinds.



A positive correlation was observed between phenolic concentration and antioxidant activity, suggesting that phenolic compounds are major contributors to free radical scavenging activity.

Discussion

The present study establishes that wild mushrooms from Chhattisgarh represent a rich reservoir of antioxidant compounds. The exceptional antioxidant activity of *T. heimii* may be attributed to its elevated concentration of phenolics, flavonoids, polysaccharides, and other bioactive metabolites. Phenolic compounds function as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators, thereby contributing significantly to antioxidant defence mechanisms. The observed antioxidant efficacy of these mushroom species is primarily attributed to their rich phenolic and flavonoid profiles, which facilitate stable radical neutralization through hydrogen-atom transfer and electron-donation mechanisms[20,21].Specifically, the presence of multiple hydroxyl groups within the flavonoid structure enhances their capacity to act as effective electron donors, thereby stabilizing reactive oxygen

species[22,23]. This structural stability is further reinforced by the conjugation of double bonds and the arrangement of the catechol ring, which together permit the neutralization of free radicals via sequential proton loss electron transfer[24,25]. Furthermore, the iron-chelating capabilities observed in *T. heimii* and *G. lucidum* underscore a synergistic pathway for inhibiting lipid peroxidation, effectively halting the propagation of hydroxyl radicals generated via the Fenton reaction[26,27]. These findings align with previous research indicating that the free radical-scavenging activity of fungi is intrinsically linked to their phenolic composition and hydrogen-donating capacity[28]. These findings provide scientific validation for the traditional consumption of wild mushrooms and highlight the therapeutic significance of *Termitomycesheimii* as a valuable source of natural antioxidants.

Conclusion

The present study demonstrated substantial antioxidant potential among wild mushrooms collected from Chhattisgarh forests. Among all investigated species, *Termitomycesheimii* emerged as the most potent antioxidant-producing mushroom, exhibiting maximum DPPH scavenging activity, metal-chelating ability, and elevated phenolic and flavonoid concentrations.

The study highlights the importance of indigenous mushroom biodiversity as a source of bioactive compounds and supports the utilization of *T. heimii* for nutraceutical and pharmaceutical applications. Further purification and characterization of antioxidant metabolites may facilitate the development of novel natural antioxidant products. In summary, the inherent ability of these compounds to act as radical terminators within various oxidative systems suggests significant potential for their application as natural dietary supplements or pharmaceutical agents. Moreover, these compounds function by terminating oxidative chain reactions in lipid-based matrices, thereby providing substantial protection against oxidative degradation in food systems. This dual functionality—extending both to the preservation of lipid stability and the mitigation of cellular-level oxidative stress—highlights the therapeutic relevance of these polyphenols in combating metabolic diseases associated with free radical damage. The therapeutic potential of these agents is further reinforced by their proven ability to inhibit microbial growth and modulate mutagenic pathways, positioning them as versatile candidates for multi-faceted medicinal interventions

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