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Antimicrobial and Antioxidant Potential of Methanolic Extracts of Wild *Agaricus bisporus* from Mahendragiri Hills, India

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ABSTRACT

The study assessed the antimicrobial and antioxidant activities of the methanolic extract from *Agaricus bisporus* gathered from Mahendragiri Hills, Andhra Pradesh, India. Preliminary screening for phytochemicals confirmed the presence of bioactive compounds such as phenols, flavonoids, alkaloids and glycosides, showing strong potential for pharmacological use. The extract demonstrated significant antibacterial activity against five bacterial strains. The highest zone of inhibition measured 24.0±0.7 mm against *Klebsiella pneumoniae* at 200 µg/ml. The lowest inhibition was 10.0±0.2 mm against *Pseudomonas aeruginosa* at 50 µg/ml. Antifungal tests indicated maximum inhibition of 20.0±0.5 mm against *Aspergillus fumigatus* and 18.3±0.6 mm against *A. niger*, both at 200 µg/ml, suggesting broad antifungal potential. Antioxidant effectiveness was measured using DPPH, reducing power and total antioxidant capacity (TAA) assays. The extract demonstrated strong DPPH radical scavenging ability, with an IC... € of 0.0660 µg/ml and a TAA value of 75.5 µg. Although its reducing power 0.09498 absorbance was lower than BHT at 0.1076 and ascorbic acid at 0.1965, it still showed notable electron-donating ability. These results showed that wild *A. bisporus* was a promising source of natural antioxidants and antimicrobial agents, which may be developed into functional foods or therapeutic products.

Key words: Antimicrobial activity, antioxidant assay, DPPH, reducing power assay, total antioxidant capacity, phenolic compounds

INTRODUCTION

Mushrooms have long been valued for their nutritional and medicinal benefits. Ancient civilizations, including the Swedish, Greek, Egyptian, Roman and Japanese cultures, used mushrooms for healing purposes. Today, they are increasingly researched for their possible health benefits. Many mushrooms show antimicrobial, immune-supporting and antioxidant effects. They are rich in proteins, minerals and bioactive compounds, making them important not only as food but also as natural sources of therapeutic agents. Recently, concerns about the safety and long-term use of synthetic antioxidants in food and pharmaceuticals have led to a movement toward natural alternatives. Mushroom-derived antioxidants, such as phenolics and flavonoids, can neutralize harmful free radicals. This reduces oxidative stress and the risk of chronic diseases. Their ways of working include binding metal ions, stopping chain reactions, breaking down peroxides and scavenging reactive oxygen species. Natural antioxidants are usually seen as safer as and more compatible than synthetic ones.

Wild mushrooms are being studied for their increased bioactive potential. Environmental stressors in their natural habitats often lead to the production of secondary metabolites that offer antioxidant and antimicrobial benefits. Studies by Raghupathi *et al*. (2018) and Thakare *et al*. (2024) have shown strong antioxidant activities in wild mushrooms from India's Western Ghats. *Chlorophyllum molybdites* has also been recognized as a strong source of antimicrobial volatile compounds (Ramarao *et al*., 2020). These findings highlight the health benefits of wild mushroom species. Among edible mushrooms, *Agaricus bisporus* is well-known and widely eaten. These have

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unique chemical profiles, likely shaped by their growing conditions. These wild varieties are rich in phenolic compounds, flavonoids and antioxidants that help them to fight against free radicals. This has implications for preventing diseases related to oxidative stress. In addition to their antioxidant properties, wild *A. bisporus* has shown antimicrobial activity against different pathogens. This is due to bioactive compounds like terpenoids and alkaloids. Even though the cultivated version is popular worldwide, wild variants of *A. bisporus* remain largely unexplored. Studying their antioxidant and antimicrobial properties could provide new insights into natural therapeutic agents. It could also support the development of functional foods and pharmaceutical applications based on wild fungal diversity. This study aimed at examining the antimicrobial and antioxidant potential of wild *A. bisporus*.

MATERIALS AND METHODS

A. bisporus was collected and taxonomically identified by the Department of Botany, Andhra University, Visakhapatnam, in November 2023 from the Mahendragiri Hills region (18°582 143 N**,** 84°222 043 E), located in Srikakulam district, Andhra Pradesh, India.

Dried and powdered mushroom samples (20 g) were subjected to Soxhlet extraction using 200 ml of methanol as the solvent. The extraction was carried out for 2 to 5 h at a temperature not exceeding the boiling point of the solvent. Following extraction, the solution was filtered, and the filtrate was concentrated to dryness. The resulting extract was stored in glass vials at 4°C. A stock solution (100 mg/ml) was prepared by dissolving the dried extract in 25% aqueous dimethyl sulfoxide (DMSO). The extracts were stored at -18°C until further use. For bioassays, the extracts were re-dissolved in 5% DMSO.

Preliminary biochemical tests for the presence of carbohydrates, proteins, alkaloids, glycosides, steroids, phenols, resins, tannins, quinones and flavonoids were carried out on the crude methanolic extract using standard procedures. The presence of different biomolecules was identified based on specific biochemical reactions.

The microbial strains used in this study were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains included *Staphylococcus aureus* (MTCC 3160), *Streptococcus pyogenes* (MTCC 442), *Klebsiella pneumoniae* (MTCC 452), *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 424). These bacteria were cultured on freshly prepared nutrient agar and incubated at 37°C for 24 h. The fungal strains included *Aspergillus niger* (MTCC 961), *Aspergillus flavus* (MTCC 3396) and *Aspergillus fumigatus* (MTCC 2584). These were grown on potato dextrose agar and incubated under appropriate conditions for fungal growth.

To test for antibiotic activity, the bacterial and fungal cultures were incubated overnight at 37°C and 28°C. Separate 100 ml conical flasks containing the nutrient agar medium and potato dextrose agar medium were autoclave sterilized at 121 °C and 15 Lbp for 15 min before the media were transferred into Petri plates. Using a sterilized glass spreader, the bacterial and fungal inoculums were applied to the agar plate surface. Using a sterile cork borer, four antibacterial and antifungal wells were created at identical distances. When the zone of inhibition was larger than 8 mm, antibacterial activity was observed. Three duplicates of each study were conducted and the mean value was determined. Sterilized streptomycin was employed as the control antibiotic.

Methanol was used to prepare diluted working solutions of the test extracts. Precisely 100 µl of each test sample (ranging from 0.6 to 20.0 mg/ml in methanol) was mixed with 5 µl of a 0.002% DPPH solution in methanol in 96-well microtiter plates. To evaluate the antioxidant activity, 1 ml of the DPPH solution was combined with 1 ml of each sample or reference solution. The mixtures were incubated in the dark at room temperature for 20 min. The absorbance was then measured at 517 nm using a spectrophotometer, with methanol serving as the blank. A control solution consisting of 1 ml methanol and 1 ml of 0.002% DPPH was used to calculate the per cent inhibition.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH inhibition (\%)} = (A”B/A) \times 100$$

Where:

A = Absorbance of the control (blank)
B = Absorbance of the sample

The total antioxidant capacity was determined based on the reduction of Mo(VI) to Mo(V), resulting in the formation of a green phosphate/Mo(V) complex under acidic conditions. Briefly, 1 ml of extract (1 mg/ml) was mixed with 1 ml of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank. The antioxidant capacity was expressed as ascorbic acid equivalents (AAE).

Different concentrations of *A. bisporus* methanol extract were prepared, and 1.0 ml of each was mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added, and the mixtures were centrifuged at 200 × g for 10 min. Subsequently, 1.25 ml of the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml of 0.1% (w/v) ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm. Higher absorbance values indicated greater reducing power. The EC_{50} value (extract concentration required to achieve an absorbance of 0.5) was determined from the absorbance versus concentration plot. Butylatedhydroxytoluene (BHT) was used as the standard.

All experiments were performed in triplicate, and the results were presented as mean±standard deviation (SD). Zone of inhibition measurements were recorded in millimetres (mm), and average values were calculated using Microsoft Excel. For the DPPH assay, the IC_{50} value (the concentration of the extract required to inhibit 50% of DPPH radicals) was determined by plotting the percentage of DPPH scavenging activity against the extract concentrations and fitting the data to a linear regression model. The IC_{50} values were calculated from the regression equation. Statistical analyses were carried out using the Statistical Analysis System (SAS) software.

RESULTS AND DISCUSSION

A preliminary mycochemical screening of the methanolic extract of *A. bisporus* was conducted to detect various bioactive compounds using standard qualitative biochemical tests. The presence of secondary metabolites such as carbohydrates, proteins, alkaloids, glycosides, steroids, phenols, resins, tannins and flavonoids was confirmed, while quinones were absent (Table 1).

Table 1. Mycochemical constituents of *Agaricus bisporu* methanol extract

Name of the test	Results
Carbohydrates	++
Proteins	++
Alkaloids	++
Glycosides	++
Steroids	+
Phenols	+++
Resins	+
Tannins	+
Quinones	-
Flavonoids	+++

Alkaloids were detected using Dragendorff's and Mayer's reagent. This indicated their role in fighting infections and cancer because they inhibited blood vessel formation and regulated ion channels. Glycosides showed a positive result based on colour change during hydrolysis. This supported their potential for treating heart and metabolic issues. Steroids were identified by a colour change with acetic anhydride and sulfuric acid, suggesting they were important for cell membranes and hormone control. Phenolic compounds were identified with a ferric chloride test, showing a strong presence (+++), indicating powerful antioxidant activity. Similarly, flavonoids were strongly present (+++), confirmed by aluminum chloride and ammonia tests. These compounds played a role in removing free radicals, reducing inflammation and combating cancer. There was a moderate presence of proteins and carbohydrates confirmed by Biuret's, Millon's, and Molisch's tests, highlighting their nutritional benefits. Resins and tannins were weakly present (+), with functions in fighting infections and aiding wound healing, respectively. Quinones showed no response, meaning they were not found in this extract. The methanolic extract of *A. bisporus* had many important compounds, especially

phenols and flavonoids in high concentrations. These compounds are known for their strong antioxidant properties and might help with cancer prevention, aging and immune system support. The variety of bioactive substances found suggests *A. bisporus* having potential as a functional food and a source of natural medicines. The absence of quinones might lower harmful effects, making it safer for medical uses.

The methanolic extract of *A. bisporus* showed a wide range of secondary metabolites, including phenols, flavonoids, alkaloids, glycosides, steroids, tannins and resins, while quinones were not present. The strong reactions for phenolic and flavonoid compounds indicated high antioxidant potential. This aligned with previous finding that highlight *A. bisporus* as a rich source of polyphenols and flavonoids (Assemie and Abaya, 2022; Bristy *et al.*, 2022). These compounds are crucial for scavenging free radicals, managing oxidative stress and preventing chronic diseases. The identification of alkaloids and glycosides supported the mushroom's possible roles in fighting infections and regulating metabolism, while the moderate presence of steroids and tannins related to structural and protective functions (Jankov *et al*., 2024). The lack of quinones may help to reduce harmful oxidative activity, improving its safety for therapeutic or health-related uses.

The methanolic extract of *A. bisporus* showed a dose-dependent antibacterial effect against five important bacterial strains: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The extract was tested at concentrations of 50, 100, 150 and 200 µg/ml. The results as the mean zone of inhibition (in mm) ± standard deviation (SD), based on three replicates, are shown in Table 2. The antibacterial activity increased with concentration for all test organisms. The methanol extract of *A. bisporus* had concentration-dependent antibacterial activity across all tested bacterial strains. The zone of inhibition ranged from 10.0 ± 0.2 mm at the lowest concentration (50 µg/ml) to 24.0 ± 0.7 mm at the highest concentration (200 µg/ml). The strongest antibacterial effect was seen against *K. pneumoniae* at 200 µg/ml, while the weakest was observed against *P. aeruginosa* at 50 µg/ml. Among the five tested pathogens, *K. pneumoniae*, *S. pyogenes* and *S. aureus* were more susceptible to the extract. In contrast, *E. coli* and *P. aeruginosa* showed moderate to lower sensitivity. These findings indicated that the methanolic extract contained bioactive compounds that were particularly effective against both Gram-negative and Gram-positive bacteria. The results suggested that the methanol extract from *A. bisporus* had strong inhibitory effects, especially against Gram-negative *K. pneumoniae*.

Antifungal activity of the methanol extract was assessed against three pathogenic fungal strains: *Aspergillus niger* (MTCC-961), *A. flavus* (MTCC-3396) and *A. fumigatus* (MTCC-2584), using concentrations of 50, 100, 150 and 200 µg/ml. Results are presented as mean inhibition zones ± SD in Table 3 and Fig. 1. In the antifungal assay, the methanol extract of *A. bisporus* also showed increasing activity with higher concentrations. The zone of inhibition ranged from 10.2±0.3 mm at 50 µg/ml to 20.0±0.5 mm at 200 µg/ml. The highest antifungal activity was recorded against *A. fumigatus* at 200 µg/ml, followed closely by *A. niger* and *A. flavus*. The lowest activity was observed against *A. flavus* at the 50 µg/ml concentration. These results indicated that the mushroom extract possessed promising antifungal properties, with the strongest effect seen against *A. fumigatus*, a clinically important fungal pathogen. These results highlight the promising antifungal potential of *A. bisporus*, especially against filamentous fungi such as *A. fumigatus*.

The methanolic extract exhibited concentration dependent antibacterial and antifungal activities against both Gram-

Table 2. Antibacterial activity of *A. bisporus* methanol extract (zone of inhibition in mm±SD)

S. No.	Name of bacterium	50 µg	100 µg	150 µg	200 µg
1.	*Staphylococcus aureus*	16.2±0.4	18.1±0.5	18.3±0.3	20.0±0.6
2.	*Streptococcus pyogenes*	12.4±0.3	16.0±0.6	18.0±0.5	21.2±0.4
3.	*Escherichia coli*	10.1±0.2	12.3±0.4	14.0±0.6	18.4±0.5
4.	*Klebsiella pneumoniae*	10.5±0.3	15.2±0.5	16.4±0.4	24.0±0.7
5.	*Pseudomonas aeruginosa*	10.0±0.2	12.1±0.3	14.2±0.5	18.0±0.6

Table 3. Antifungal activity of *A. bisporus* methanol extract (zone of inhibition in mm ± SD)

S. No.	Name of bacterium	50 µg	100 µg	150 µg	200 µg
1.	*Asperigillus niger*	12.0±0.5	14.1±0.3	16.2±0.4	18.3±0.6
2.	*Asperigillus fumigatus*	12.4±0.4	14.0±0.5	18.0±0.6	20.0±0.5
3.	*Asperigillus flavus*	10.2±0.3	12.3±0.4	18.1±0.5	18.0±0.7

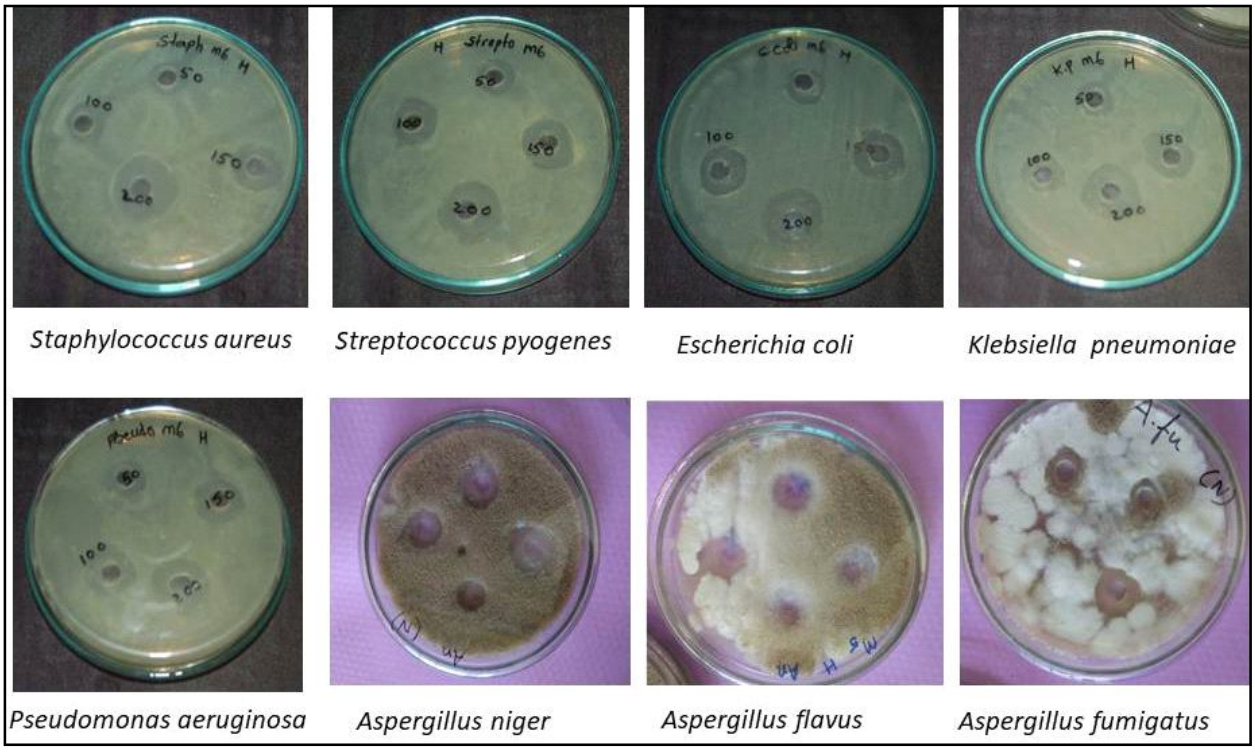

Fig. 1. Different observations of antimicrobial activity of *A. bisporus* against various bacterial and fungal pathogens.

positive and Gram-negative organisms. Among bacteria, *K. pneumoniae* showed the highest susceptibility, followed by *S. pyogenes* and *S. aureus*, consistent with previous findings demonstrating similar inhibitory patterns of *A. bisporus* extracts against these pathogens (Karnwal, 2020; Jankov *et al*., 2024). The antifungal efficacy observed against *A. fumigatus*, *A. niger* and *A. flavus* also aligned with studies reporting the fungicidal potential of *A. bisporus* extracts and isolated metabolites (El-Maradny *et al*., 2025). The antimicrobial effects are largely attributed to the synergistic action of phenolic and flavonoid compounds that can disrupt microbial cell walls, inhibit enzymatic activity and interfere with nucleic acid synthesis (Martins *et al*., 2023; Babu *et al*., 2024). The variation in zone diameters among tested strains may be due to differences in cell wall structure and permeability between Gram-positive and Gram-negative organisms.

The antioxidant potential of the methanolic extract of *A. bisporus* was evaluated using three standard assays: DPPH free radical scavenging activity, reducing power assay and total antioxidant capacity. These tests provided a comprehensive understanding of the extract's ability to neutralize free radicals and reduce oxidative stress.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely accepted method for evaluating the free radical scavenging efficiency of bioactive compounds. In this assay, the stable nitrogen-centered DPPH radical, which exhibits a deep violet colour, is reduced to a yellow-coloured diphenylpicrylhydrazine upon accepting an electron or hydrogen atom from an antioxidant compound. The methanolic extract of *A. bisporus* showed a concentration dependent scavenging activity, with increasing inhibition of the DPPH radical observed as the extract concentration increased (Table 4).

Table 4. DPPH radical scavenging activity of *A. bisporus* methanol extract

S. No.	Concentrations	Reading
1.	50 µl	52.75
2.	100 µl	73.92
3.	150 µl	80.13
4.	200 µl	92.04
5.	IC_{50}	0.0660

The scavenging activity ranged from 52.75% at 50 µl to 92.04% at 200 µl, indicating strong antioxidant potential. The IC_{50} value the concentration required to inhibit 50% of the DPPH radicals was found to be 0.0660 µg/ml (Fig. 2). A lower IC_{50} value corresponded to higher free radical scavenging efficiency, suggesting that the methanolic extract of *A. bisporus* was a potent natural source of antioxidants.

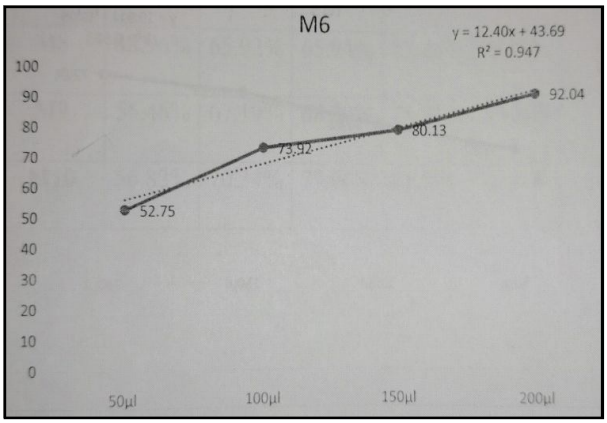

Fig. 2. *A. bisporus* DPPH reducing activity scattered plot.

The reducing power assay is based on the principle that antioxidants in the sample can donate electrons, converting ferric ions ($Fe^{3}z$) into ferrous ions ($Fe^{2}z$), thereby reducing the yellow-coloured ferric complex to the blue-coloured ferrous form. This transformation can be quantitatively measured by the formation of Perl's Prussian blue complex, with increased absorbance at 700 nm indicating greater reducing capacity. In the current study, the methanolic extract of *A. bisporus* exhibited moderate reducing power when compared to standard antioxidants such as ButylatedHydroxytoluene (BHT) and ascorbic acid (Table 5).. The extract showed an absorbance value of 0.09498, which was lower than both BHT (0.1076) and ascorbic acid (0.1965). This suggests that while the mushroom extract possesses reducing ability due to its hydrogen-donating potential, it is relatively weaker than synthetic and natural antioxidant standards.

Table 5. Absorbance values of reducing power assay of *A. bisporus*

S. No.	TAA values	Reducing power assay values (RPA)
01.	*A. bisporus*	0.09498
02.	BHT	0.1076
03.	Ascorbic acid	0.1965

The total antioxidant capacity of the extract was assessed using the phosphomolybdenum method. This method relies on the reduction of Mo (VI) to Mo (V) by antioxidant compounds, resulting in a green-coloured phosphate/Mo (V) complex. This complex shows maximum absorbance at 695 nm. The methanolic extract of *A. bisporus* had a TAA value of 75.5 µg/ml, indicating strong total antioxidant activity. High absorbance and TAA values show a greater ability of the sample to neutralize reactive oxygen species (ROS). This strong antioxidant potential can help suppress diseases related to oxidative stress, such as aging, cancer and neurodegenerative disorders. These findings highlight the potential of *A. bisporus* as a valuable source of natural antioxidants and suggest its use in developing functional foods or therapeutic agents.

The extract also showed strong antioxidant activity in several assays, including DPPH, reducing power and total antioxidant capacity tests. The high DPPH radical-scavenging percentage of 92.04% at 200 µl and low IC_{50} value of 0.066 mg/ml indicated a significant ability to neutralize free radicals. This supported earlier studies that reported IC_{50} values between 0.03 and 0.19 mg/ml for *A. bisporus* methanolic extracts (Bristy *et al.*, 2022; El-Maradny *et al.*, 2025). The moderate reducing power and significant total antioxidant capacity of 75.5 µg/ml further confirmed its effectiveness as an electron donor, comparable to other edible mushrooms known for their antioxidant potential. Overall, these results indicate that *A. bisporus* had strong bioactive potential due to its phenolic and flavonoid components, supporting its development as a functional food ingredient or natural therapeutic agent.

CONCLUSION

This study showed that the methanolic extract of wild *Agaricus bisporus*, collected from the Mahendragiri Hills region of Andhra Pradesh,

had significant antimicrobial and antioxidant activities. The extract displayed a concentration dependent inhibitory effect against various bacterial pathogens. The highest zone of inhibition occurred against *Klebsiella pneumoniae* (24.0±0.7 mm at 200 µg/ml) and substantial antifungal activity, particularly against *Aspergillus fumigatus* (20.0±0.5 mm at 200 µg/ml). These results imply the presence of potent bioactive compounds that can target both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi. Phytochemical screening confirmed the presence of important secondary metabolites, including phenols, flavonoids, alkaloids, glycosides and tannins, likely responsible for the observed biological activities. The extract also exhibited strong antioxidant potential, shown by high DPPH radical scavenging activity of 92.04% at 200 µl and a low IC_{50} value of 0.0660 µg/ml. Additionally, the total antioxidant capacity of 75.5 µg/ml and moderate reducing power further established its potential as a natural source of antioxidant agents. *A. bisporus* showed promising pharmacological potential and may serve as a valuable candidate for developing natural antimicrobial and antioxidant agents in nutraceutical and pharmaceutical applications. Further studies on compound isolation and mechanism analysis are needed to fully explore its therapeutic value.

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