

EVALUATING THE THERAPEUTIC EFFICACY OF TWO CULINARY MUSHROOMS: A COMPARATIVE STUDY ON THE ANTIOXIDANT AND ANTICANCER ACTIVITIES OF *PLEUROTUS ERYNGI* AND *HYPsizYGUS MARMOREUS*

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ABSTRACT

The present study was conducted to assess the phytochemical profile, antioxidant activity and anticancer activity of the most widely consumed culinary mushrooms *Pleurotus eryngi* and *Hypsizygyus marmoreus*. Molecular identification of the species has been done by sanger sequencing technique. Qualitative analysis authenticated the presence of necessary phytochemicals including glycosides, phenolics, alkaloids, terpenoids. The ethanolic extract of the mushrooms exhibited significant antioxidant activity in a concentration dependend manner as observed from DPPH, ABTS, FRAP and NO radical scavenging assays. Anticancer efficiency of the samples was tested using MTT assay on MCF -7 cell line. The extract produced cell cytotoxicity in a dose-dependent pattern within biologically safe levels, indicating its potential application as a cancer-preventive agent or in complementary therapy.

Keywords: *Hypsizygyus marmoreus*, *Pleurotus eryngi*, Antioxidant activity, Cytotoxicity, Phytochemicals.

INTRODUCTION

Mushrooms have been widely consumed in India, especially during the monsoon season, because of their high nutritional value and medicinal benefits (Kumar *et al.*, 2022). *Hypsizygyus marmoreus* is known for its high concentration of beneficial phytochemicals, including polysaccharides, proteins, phenolics, and flavanoids. Considering a study report, water-soluble extracts of *H. marmoreus* have demonstrated anticancer properties by inhibiting the proliferation of various cancer cell lines (Bao *et al.*, 2011). The antioxidant activity of *Hypsizygyus marmoreus* can be associated with the high content of phenolic compounds, which are well known for free radical-scavenging and management of oxidative stress-induced cell damage and effective in prevention of many chronic disorders associated with oxidative stress, like cardiovascular diseases and neurodegenerative diseases (Bach *et al.*, 2019). A study conducted by Chang *et al.*, in 2004 reported that whole *H. marmoreus* extracts inhibited the proliferation of HepG2 cells, also purified a thermo stable protein named hypsin from *H. marmoreus*, which

exhibited strong antiproliferative activity against HepG2 cells. On interpretation of these available results, the role of naturally occurring antioxidants in various *H. marmoreus* strains and their role in preventing cancer is yet to be explored. The majority of previous research has concentrated on polysaccharide fractions, which are not only antioxidant and anti-inflammatory but also a promising candidate for cancer therapy. *Pleurotus eryngii*, apart from its excellent taste and flavour, represents one of the most commercially important species in the world. *Pleurotus eryngi* is known for the presence of many biologically active substances such as polysaccharides, lipids, peptide, sterols and dietary fibres (Liu, *et al.*, 2010). Reports suggests the presence of polysaccharides and has good therapeutic effects, such as its ability to lower cholesterol, promote intestinal digestion, prevent cardiovascular disease and improve immunity (Zhang *et al.*, 2020). The presence of 1,6-branched and 1,3- β glucans have been reported in *P. eryngi* which inhibits tumor growth by stimulating the immune system through activation of macrophages and subsequent effects on natural killer (NK),

cells and cytokine production (Hetland *et al.*, 2011). Reports also implicate polysaccharides with varying sugars such as beta- and alpha-glucans. (Borchers *et al.*, 2008). Such mushroom polysaccharides are now evaluated as adjuvant cancer therapy compounds alongside conventional cancer treatments (Standish *et al.*, 2008).

As per reports, *Pleurotus eryngii* contains a wide variety of antioxidant compounds like phenolic acids, flavonoids, polysaccharides, vitamin C and E and ergothioneine and exhibits significant antioxidant activity in vitro using assays such as DPPH, ABTS, NO and FRAP (Kim *et al.*, 2006). Due to its potent antioxidant properties and safety as a food source, *Pleurotus eryngii* has been considered a promising functional food with nutraceutical applications for improving human health. Literary data suggests that these widely consumed culinary mushrooms are good sources of medicinal compounds and its potential in the context of anticancer activity has not been extensively explored. This study focusses on qualitative phytochemical profiling along with in-depth assessment of antioxidant potential and invitro cytotoxic potential of the collected samples.

MATERIALS AND METHODS

Sample Collection

The mushrooms *Pleurotus eryngii* and *Hypsizygus marmoreus* were collected from Vidyaranya pura, Bengaluru, Karnataka

Molecular characterization

DNA isolation of the collected samples has been done using Nucleosieve fungal DNA isolation Kit purchased from Primordia Lifesciences, Kalamassery, Ernakulam. PCR amplification was done using ITS primers and sequenced using sanger sequencing technique. The sequence identity confirmed using NCBI BLAST search.

Chemicals

All the chemicals and biochemicals used in the present study were purchased from Merck and the solvents used are of analytical grade from Merck. Phosphate Buffered Saline (PBS) and Dulbecco's Modified Eagle Medium (DMEM), high glucose medium, penicillin/streptomycin and fetal bovineserum (FBS) were purchased from Sigma Aldrich and Gibco. 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent and Dimethyl sulfoxide (DMSO) were procured from Hi-media.

Extraction

A total of 500 g of the mushrooms were used for the extraction process. The mushroom samples were washed twice with double distilled water to remove surface impurities and dried in room temperature. The mushrooms were chopped and grinded using a sterile mortar and pestle

and immersed in 70% ethanol and subjected to ultrasonication for 24 h to enhance solvent penetration and extraction efficiency. The extracts were then filtered and rotary evaporated for concentration. The extracts were then stored at 4 degree celcius.

Phytochemical analysis

Screening for glycosides, alkaloids, terpenoids, tannins/polyphenol, flavanoids and saponins were done using the conventional standard protocol.

Invitro antioxidant activity

DPPH: The DPPH activity was measured as described by Blois (1958).

FRAP: The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996).

ABTS: Radical scavenging assay was performed according to the method of Pellegrini *et al.*,1999

NO: The NO scavenging assay was carried out according to the method of Panda *et al.*,2009.

Cell culture

Human Breast cancer cells- MCF-7 cells were obtained from NCCS, Pune. It was cultured in DMEM media supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C in a humidified atmosphere of 5% CO₂.

Invitro Anti-cancer activity

The effect of *Pleurotus eryngii* (PE) extract on MCF-7 cell proliferation was evaluated using the MTT assay at the extract concentrations varying in the range 12.5µg/µl to 200µg/µl.

Statistical analysis

Results were calculated and expressed as Mean ± Standard deviation. The data obtained in the studies were subjected to one-way of analysis of variance (ANOVA) for determining the significant difference. A *p-value* < 0.05 was considered to be significant.

Molecular Characterization

Genetic identity of the mushrooms was confirmed by performing molecular characterization through DNA isolation, PCR amplification and sequencing and similarity search using NCBI BLAST.

RESULTS AND DISCUSSION

The yield of extract per 500 gram of sample is summarized in Table 1. The qualitative phytochemical screening of *P.eryngii* detected the presence of alkaloids, polyphenols, saponins, terpenoids and glycosides. The results are summarized in Table 2. In Figure 5, the concentration dependent cytotoxicity of the extract has been represented graphically. The results indicated a dose-dependent

decrease in cell viability compared to the control (Figure7). At the lowest concentration (12.5 µg/mL), the extract showed approximately 68% cell viability, while the highest concentration (200 µg/mL) resulted in nearly 52% cell viability suggesting that *P.eryngi* extract possesses moderate anti-proliferative activity against MCF-7cells. Hence, the assay revealed that a dose-dependent decrease in

MCF-7cell viability upon treatment with *P.eryngi* extract. However, the extract didn't achieve 50% inhibition within the tested concentration range (12.5–200 µg/mL), and the estimated IC 50 value was found to be greater than 200 µg/mL, indicating moderate cytotoxic activity against MCF-7 cells.

Table 1. Yield of extract per sample.

Mushroom	Weight of Sample (g)	Volume of extract (ml)	Weight of extract (g)	Yield of extract (%)
<i>Pleurotus eryngi</i>	500	1000	20.972	4.1944
<i>Hypsizygus marmoreus</i>	500	1000	11.102	2.22

Table 2. Qualitative phytochemical screening of *Pleurotus eryngi*.

Phytochemical analysis	Reaction	Result
Alkaloids (Dragendoff's test)	Orange red Colour formation	Presence of alkaloids
Tannin/ Polyphenol	Blue colour was observed	Presence of tannins/ polyphenols
Terpenoids	Reddish brown ring formation	Presence of terpenoids
Flavonoids	No characteristic reaction	Absence of flavonoids
Saponins	Froth formation	Presence of saponins
Steroids	No characteristic observation	Absence of steroids
Glycosides	Violet ring formation	Presence of glycosides

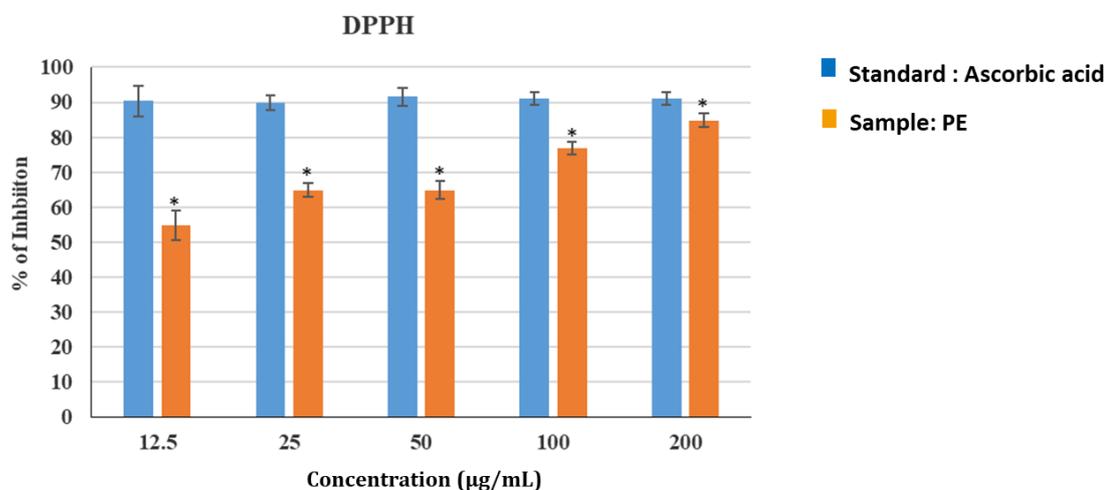


Figure 1. DPPH activity of *pleurotus eryngi* extract.

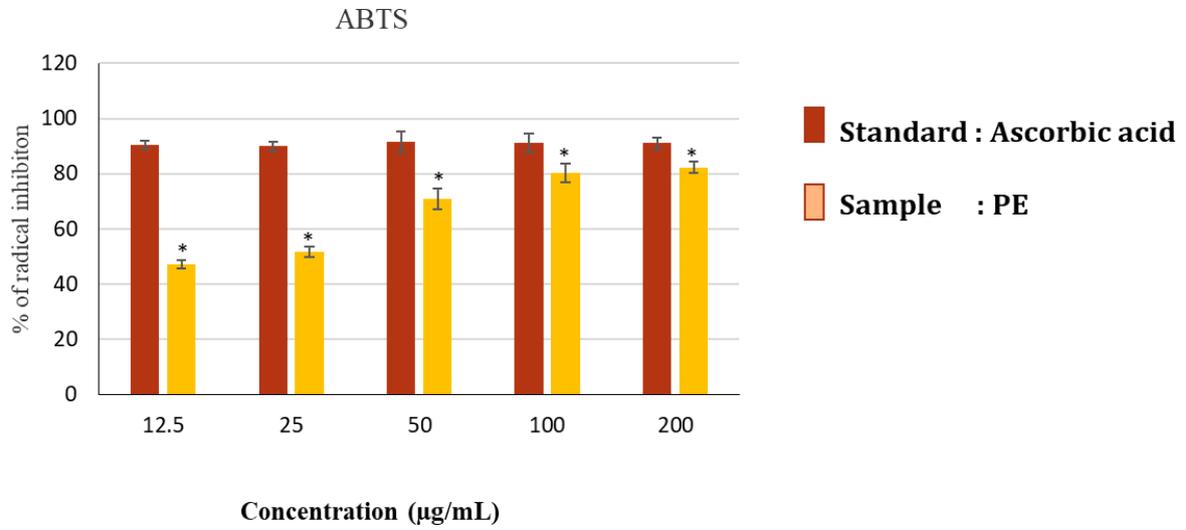


Figure 2. ABTS activity of *Pleurotus eryngi* extract

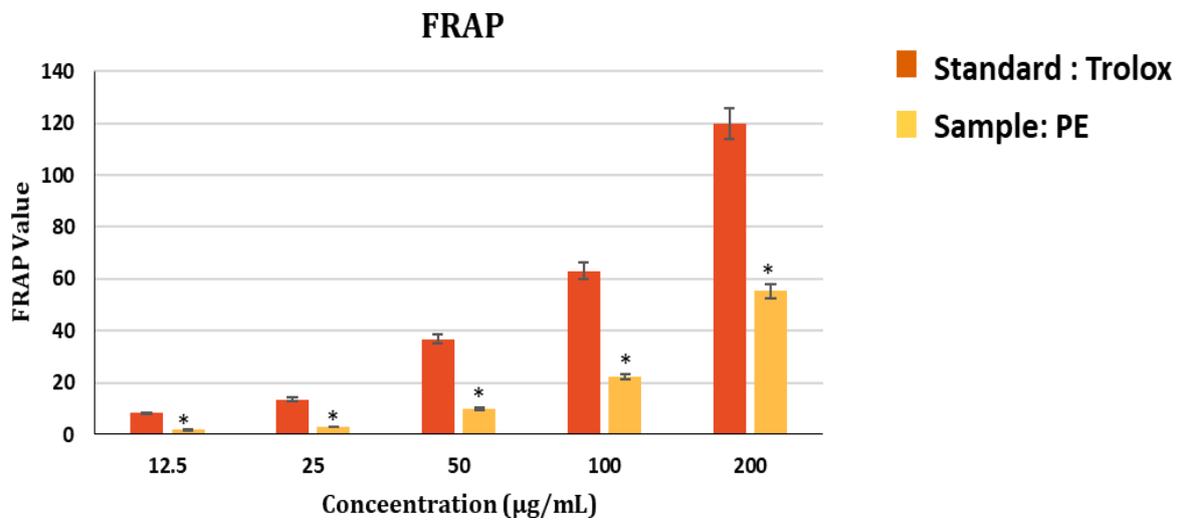


Figure 3. FRAP activity of *Pleurotus eryngi* extract

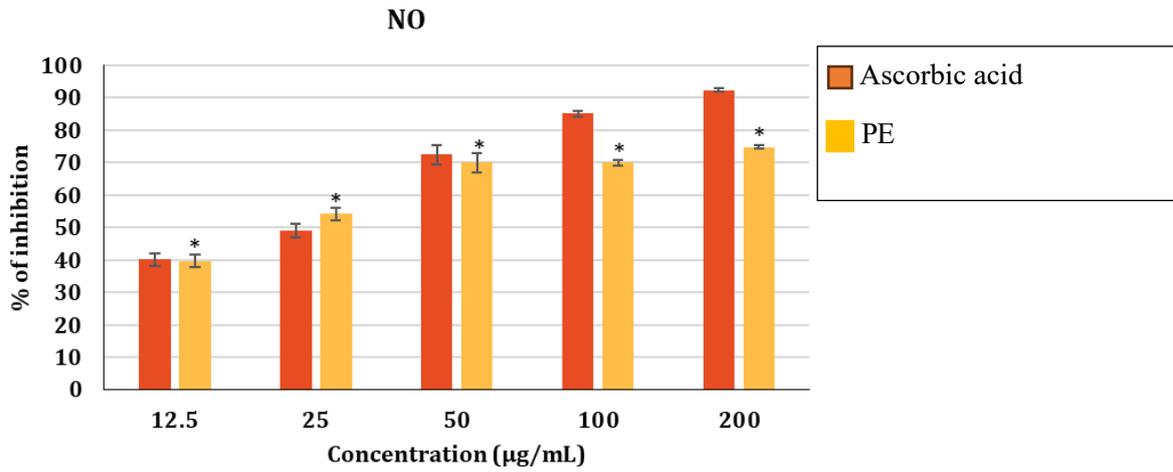


Figure 4. NO activity of *Pleurotus eryngi* extract.

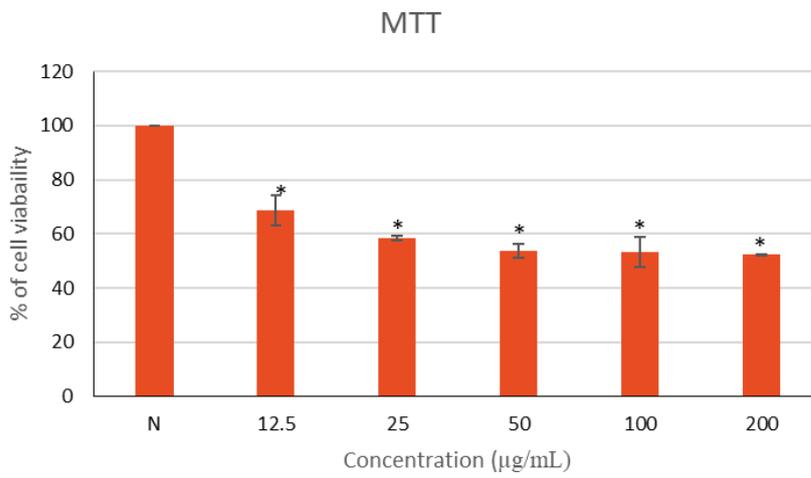


Figure 5. Cytotoxic effect of *Pleurotus eryngi* extract on MCF 7 cell line.

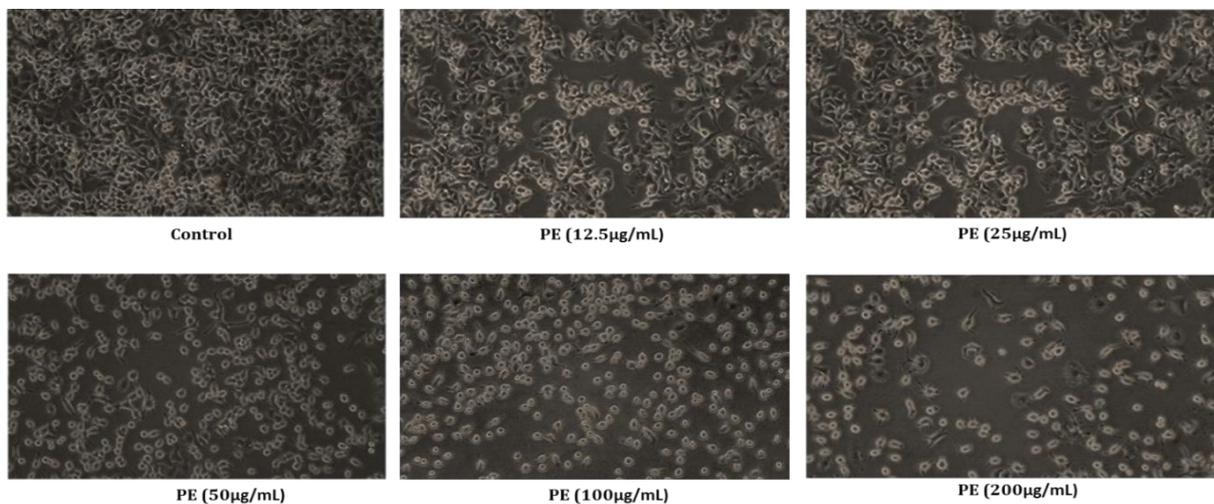


Figure 6. Phase contrast microscopic image of the cells treated with *Pleurotus eryngi* extract (Labomed, Inc., USA , magnification at 10X).

Table 3. Phytochemical screening of *Hypsizygus marmoreus*.

Phytochemical screening	Observation	Result
Test of Glycosides	Violet colour observed	Detected the presence of Glycoside
Test for Alkaloids	Orange red colour precipitate observed	Detected the presence of Alkaloids
Test for Terpenoids	Redish Brown colour observed	Detected the presence of Terpenoids
Test for Tannin/Polyphenol	Blue colour observed	Detected the presence of Polyphenol
Test for Flavanoids	No characteristic observation	Absence of Flavanoids
Test for Saponins	No characteristic observation	Absence of Saponins
Test for Steroids	No characteristic observation	Absence of Steroids
Test for Cardiac Glycosides	No characteristic observation	Absence of Cardiac glycosides

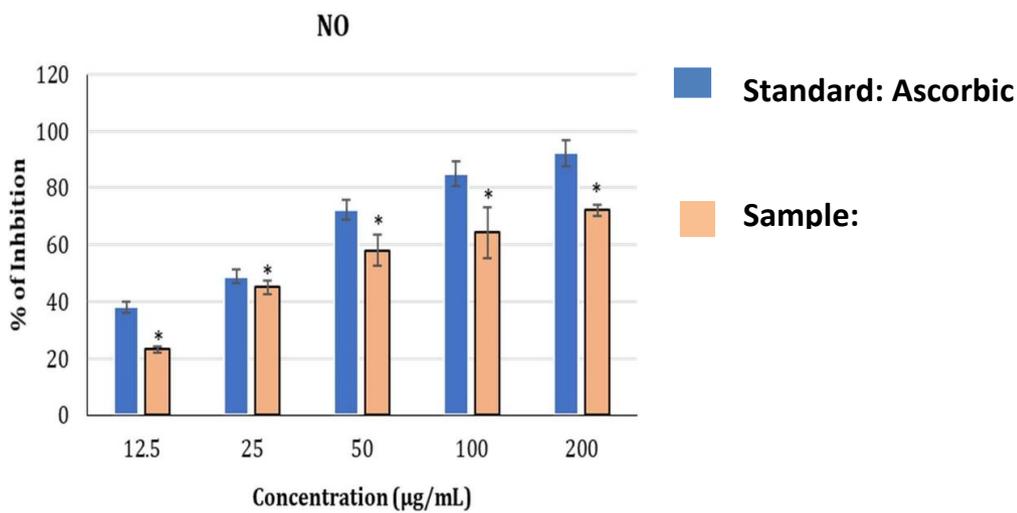


Figure 7. Showing NO activity of *Hypsizygus marmoreus* extract.

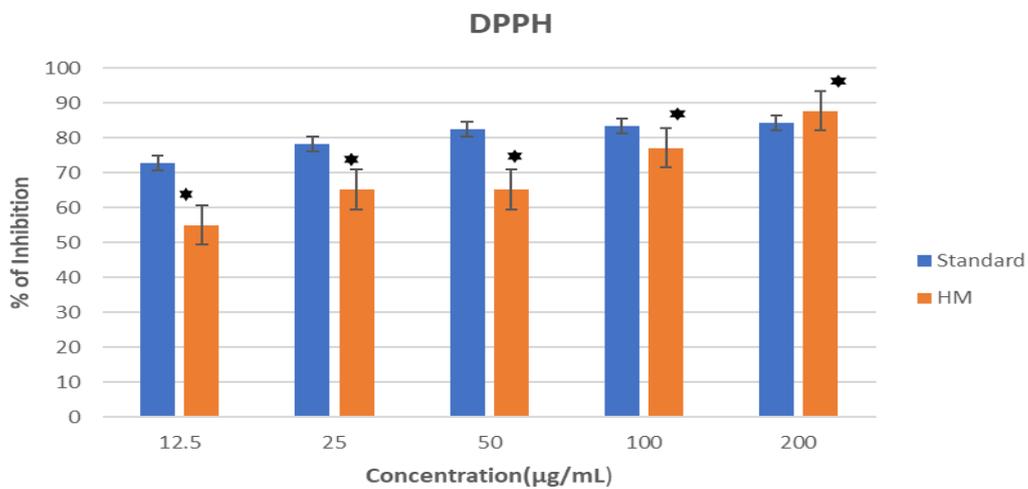


Figure 8. DPPH activity of *Hypsizygus marmoreus* extract.

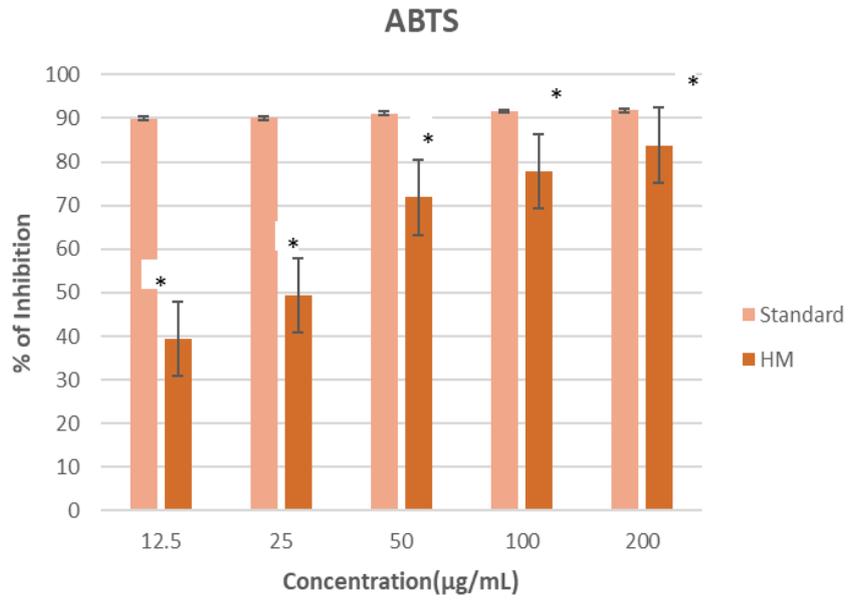


Figure 9 . ABTS activity of *Hypsizygus marmoreus* extract.

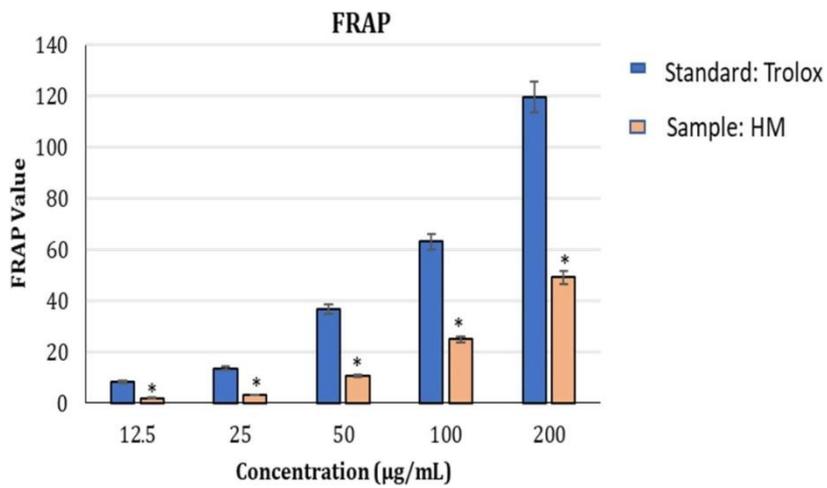


Figure 10. FRAP activity of *Hypsizygus marmoreus* extract.

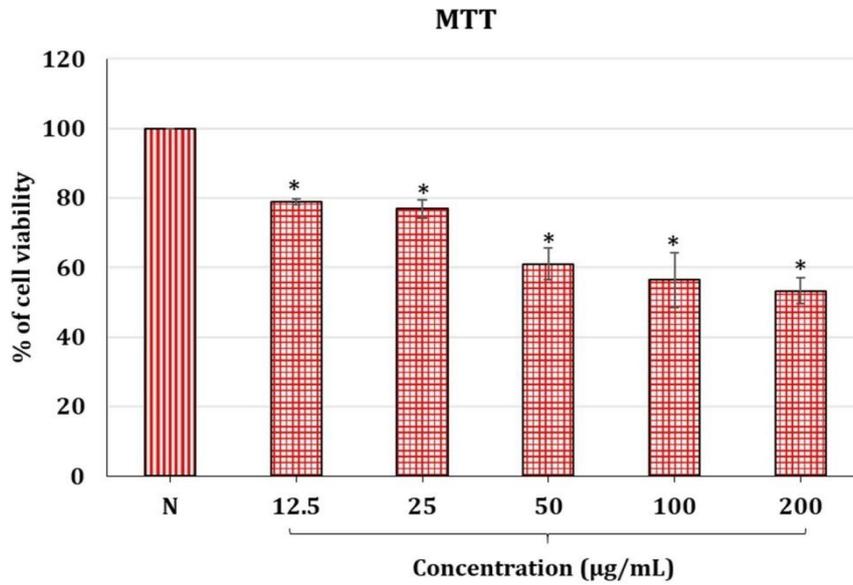


Figure 11. Cytotoxicity of *Hipsizygos marmoreus* extract on MCF -7 cell line.

The cytotoxic effect of HM extract on MCF-7 cells was evaluated using the MTT assay at concentrations of 12.5, 25, 50, 100, and 200 µg/mL (Figure 11). The results indicated a dose-dependent decrease in cell viability compared to the control group (Figure 12). At the lowest concentration (12.5 µg/mL), the extract showed approximately 78% cell viability, while the highest concentration (200 µg/mL) resulted in nearly 53% cell

viability suggesting that HM extract possesses moderate anti-proliferative activity against MCF-7 cells. Hence, the assay revealed a dose-dependent decrease in MCF-7 cell viability upon treatment with HM extract. However, the extract did not achieve 50% inhibition within the tested concentration range (12.5–200 µg/mL), and the estimated IC₅₀ value was found to be greater than 200 µg/mL, indicating moderate cytotoxic activity against MCF-7 cells.

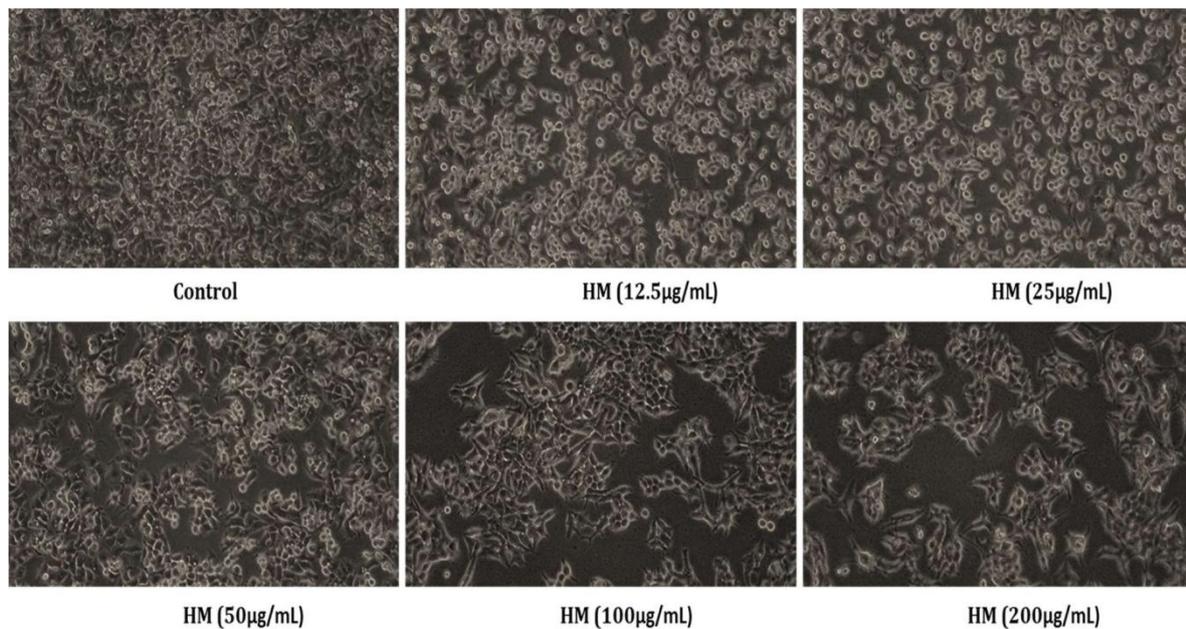


Figure 12. Phase contrast microscopic image of the cells treated with *Hipsizygos marmoreus* extract (Labomed, Inc., USA, magnification at 10X:).

The extraction yield obtained in the present study for *Hypsizygus marmoreus* (2.22%) and *Pleurotus eryngii* (4.19%) can be correlated with earlier reports indicating that ethanolic extraction efficiently elutes bioactive compounds from edible mushrooms, particularly phenolics and terpenoids (Heleno *et al.*, 2015; Ferreira *et al.*, 2009). The comparatively higher yield observed in *P. eryngii* suggests a greater abundance of ethanol-soluble metabolites compared to *H. marmoreus*. Preliminary phytochemical screening revealed the presence of glycosides, alkaloids, terpenoids, saponins, and phenolics in *P. eryngii*, while *H. marmoreus* exhibited the presence of glycosides, alkaloids, terpenoids, and polyphenols. These results corroborate with the available literature reporting that *Pleurotus* species are rich sources of secondary metabolites with strong biological activities, particularly phenolic compounds responsible for antioxidant effects (Deepalakshmi and Mirunalini, 2014; Barros *et al.*, 2008). The antioxidant potential of both mushroom extracts was confirmed through multiple *in vitro* assays. The DPPH radical scavenging activity of both *H. marmoreus* and *P. eryngii* showed strong hydrogen-donating ability, consistent with earlier reports on *Pleurotus* species (Jayakumar *et al.*, 2009). The ABTS assay further showed moderate to strong radical scavenging activity when compared to the standard antioxidant ascorbic acid. From the FRAP assay, it can be assessed that both extracts possess significant electron-donating capacity in a concentration-dependent manner, which is a key mechanism underlying antioxidant defense (Benzie and Strain, 1996). The NO scavenging assay also confirmed the ability of the extracts to neutralize reactive nitrogen species, which are implicated in inflammation and carcinogenesis (Valko *et al.*, 2007). Overall, these findings support the potential of both mushrooms as natural antioxidant sources. The antiproliferative activity of *H. marmoreus* and *P. eryngii* against MCF-7 breast cancer cells produced a concentration-dependent reduction in cell viability. Relatable cytotoxic trends have been reported for *Pleurotus* species and other medicinal mushrooms, which induce apoptosis and cell cycle arrest in cancer cells through oxidative stress modulation and mitochondrial pathways (Lavi *et al.*, 2006; Wasser, 2011). The moderate cytotoxicity observed in the present study suggests that these extracts may exert selective anticancer effects within the biologically safe profile. The stronger activity observed in *P. eryngii* may be attributed to its higher phenolic and saponin content, which are known to contribute significantly to anticancer activity (Patel and Goyal, 2012). From the study, it can be inferred that the antioxidant and antiproliferative properties observed in the present study strongly support the therapeutic potential of *H. marmoreus* and *P. eryngii* as functional food ingredients and as promising sources of natural antioxidant and anticancer agents.

CONCLUSION

The present study demonstrates that the ethanolic extracts of *Hypsizygus marmoreus* and *Pleurotus eryngii* possess significant antioxidant and moderate cytotoxic activity

along with a rich phytochemical composition. Both extracts exhibited strong free radical scavenging and reducing potential across multiple *in vitro* assays, indicating their effectiveness as natural antioxidant sources. Additionally, the dose-dependent cytotoxic effects observed against MCF-7 cell lines suggest promising anticancer potential, particularly for *P. eryngii*. Overall, these findings highlight the therapeutic value of these edible mushrooms as functional food ingredients and as potential natural agents for oxidative stress-related disorders and cancer prevention.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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