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Antioxidant and Antidiabetic Properties of Selected Mushrooms from Gujarat

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KEYWORDS
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ABSTRACT:
Mushrooms are highlighted for their nutritional richness and diverse range of bioactive compounds, pointing towards their therapeutic potential. The antioxidative dimension of mushrooms, characterized for its ability to respond harmful free radicals and improve oxidative stress. In the context of diabetes, specific mushrooms are identified as promising allies. The β-glucans present in these mushrooms show potential in enhancing insulin sensitivity. While polysaccharides and β-glucosidase inhibitors demonstrate potential in regulating blood glucose levels. Inspite of these promising findings, this research paper promotes caution and highlights the necessity for further research to validate the therapeutic potential of mushrooms across a broader spectrum of health. This study provides a comprehensive overview of the health attributes associated with antioxidative defense mechanisms and potential implications for diabetes management of selected mushroom species. This reflects the ongoing nature of research in understanding the multifaceted health benefits associated with mushrooms.

Introduction:

Mushrooms, a diverse and intriguing group of organisms, have garnered significant attention for their potential health benefits, particularly for antioxidant and antidiabetic properties. This introduction delves into the antioxidant and antidiabetic aspects of selected mushrooms, shedding light on the compounds and mechanisms that underscore their therapeutic potential. Mushrooms, renowned for their nutritional richness, boast an array of antioxidants pivotal in mitigating oxidative stress. Exploring the antidiabetic potential of mushrooms reveals promising avenues for diabetes management. β-glucans, prevalent in species like *Pleurotus ostreatus*, *Lentinus sajor-caju*, *Agaricus bisporus, Ganoderma lucidum, Xylaria polymorpha, Termitomyces heimii,* and *Cordyceps militaris*, exhibit the ability to improve insulin sensitivity and regulate blood sugar levels. Notably, some mushrooms contain compounds that act as β-glucosidase inhibitors, influencing carbohydrate digestion and aiding in the maintenance of balanced blood glucose levels. Mushrooms should be considered as part of a holistic approach to health and wellness, and individuals with diabetes or other health conditions should consult healthcare professionals before incorporating significant dietary changes.

Materials and Methodology

Antioxidant Properties:

DPPH free radical scavenging activity*:* The methodological analysis as outlined by (Gülçin et al., 2010) was somewhat modified to evaluate the DPPH free radical scavenging capacity of the mushroom extracts. DPPH, in its radical form, absorbs light at 517 nm; however, when it is reduced by an antioxidant or radical species, its absorbance decreases. A 0.5 ml of 0.1 mM ethanolic DPPH solution were added to 1.5 ml of 0-100 µg/ml concentration ranges of aqueous mushroom extracts. After 30 minutes, the absorbance was measured at 517 nm against blank samples. DPPH free radical scavenging activity is influenced by reaction mixture. The DPPH scavenging capacity was expressed in mM

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within the reaction medium and calculated from the calibration curve obtained through linear regression (R^2: 0.9845) using DPPH standard curve.

$$Absorbance = 9.692\ x\ [DPPH] + 0.215 \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(1)$$

Using the following formulae the ability to scavenge the DPPH radical was calculated:

$$Inhibition\ (\%) = \frac{[A_{Control} - A_{Sample}]}{A_{control}}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.(2)$$

Where "$A_{control}$" is the absorbance of the DPPH solution as control and "A_{sample}" is the absorbance of sample (mushroom extracts).

***Hydrogen peroxide scavenging activity**:* Following the procedure as mentioned by (Ruch' et al., 1989), the hydrogen peroxide scavenging assay was performed. H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Aqueous mushroom extracts at the 0-100 µg/ml concentrations in 3.4 ml of phosphate buffer were added to each 0.6 ml of 43 mM H_2O_2 solution. The blank solution contained the sodium phosphate buffer without H_2O_2. The absorbance value was measured at 230 nm. The concentration of H_20_2 (mM) in the assay medium was quantified using a standard curve (R^2: 0.9895). The scavenging activity was determined similarly to the method of (Ruch' et al., 1989) as shown in Graph 6 and compared with BHA as standard.

$$Absorbance = 0.038\ x\ [H_2O_2] + 0.4397 \ldots\ldots\ldots\ldots\ldots\ldots Eq.(3)$$

The percentage values of H_2O_2 scavenging activity of mushroom extracts and standard compound was calculated using the following equation:

$$Inhibition\ (\%) = \frac{[A_{Control} - A_{Sample}]}{A_{control}}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.(4)$$

Nitric Oxide Radical Scavenging Assay: Mushroom samples were prepared from 0.1 ml aqueous and serially diluted with distilled water to achieve varying concentrations of 0-100 µg/ml for each extract and gallic acid. The Griess reagent was prepared by mixing 1:1 v/v proportion of 1% $C_6H_8N_2O_2S$ and 0.1% ($C_{12}H_{16}Cl_2N_2$), both in 2.5% phosphoric acid. A volume of 0.5 ml of 10 mM sodium nitroprusside in phosphate-buffered saline was mixed with 1 ml of the mushroom extract at various concentrations (0-100 µg/ml) and incubated at 25°C for 180 minutes. Each mixture was then combined with an equal volume of freshly prepared Griess reagent. Additional samples containing drugs at the same concentrations without sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) were also prepared and measured at 546 nm. The percentage inhibition and nitrite radical scavenging activity of the extracts and standard were calculated using the following formula:

$$Inhibition\ (\%) = \frac{[A_C - A_S]}{A_c}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.(5)$$

***$ABTS^+$ Scavenging capacity assay**:* The $ABTS^+$ decolorization assays were carried out as previously described (Ilyasov et al., 2020; Murrant & Reid, 2001). It involves the liberation of $ABTS^+$ chromophore by oxidation of $C_{18}H_{18}N_4O_6S_4$ with $K_2S_2O_8$. The $ABTS^+$ radical cation was formed by reacting 7 mM stock solution of ABTS with 2.45 mM $K_2S_2O_8$ and incubate to avoid direct exposure to light at room temperature for more than 6 hours before use. After mixing different concentrations of the Mushroom extracted (final concentration as 10, 15, 20% v/v) absorbance at 734 nm was measured after 10 min of incubation with 1 ml of $C_{18}H_{18}N_4O_6S_4$ solution. The percentage inhibition (Dose-dependent) was calculated from the formula:

$$Inhibition\ (\%) = \frac{[A_0 - A_S]}{A_0}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.(6)$$

***Reducing Power Assay**:* The reducing power of the mushroom extract was evaluated according to the method standardized by (Murrant & Reid, 2001). Mushroom extracts with final concentration of 10, 15, 20% v/v, BHT (0.5 mg/ml), and BHA (0.5 mg/ml) were mixed with 2.5 ml of 0.2 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% w/v potassium ferricyanide [$K_3Fe(CN)_6$]. This was then incubated for 20 min at 50°C temperature. 2.5 ml of 10% w/v TCA ($C_2HCl_3O_2$) was added to each mixture, and then centrifuged at 1000 rpm or 112 G-force (rcf) for 10 min. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water in 1:1 ratio and 0.5 ml of $FeCl_3$ (0.1% w/v) was added.

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The absorbance was measured at 700 nm. BHA (0.0-0.4 mg/ml with a difference of 0.05 mg/ml) was used as the standard (Figure 8) and the reducing power was quantified as BHT/BHA equivalent per 100 gm of mushroom.

Lipid Peroxidation Inhibition Assay: A modified Thiobarbituric acid ($C_4H_4N_2O_2S$) reactive species (TBARS) assay was used to measure lipid peroxidation. Standard BHA in the concentration range 0-0.5 mg/ml and 0.1ml of each mushroom extracts were mixed in each test tubes and the final volume was adjusted to 1ml by adding deionized water. Finally, 0.05ml of 0.07M $FeSO_4$ was added to 1 ml of mixture and incubated for 30 min to induce lipid peroxidation in each tube. Further, 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% TBA ($C_4H_4N_2O_2S$), and 0.05ml of 20% TCA ($C_2HCl_3O_2$) were added, the mixtures were than vortexed and heated for 60 min in a boiling water bath. 5ml of n-butanol was added after cooling the reaction mixture and centrifuged at 3000 rpm or 1008 G-force (rcf) for 10 min. The absorbance of the supernatant was measured at 532 nm (Ohkawa et al., 1979). The percentage inhibition (Dose-dependent) was calculated as equation 7:

$$Inhibition\ (\%) = \frac{[A_0 - A_S]}{A_0}\ x\ 100 \ldots\ldots\ldots\ldots\ldots Eq.\ (7)$$

Antidiabetic properties:

α-Amylase Inhibition Assay: The inhibition of amylase activity was performed as mentioned (Kwon et al., 2008). The different fractions of each mushroom extract viz. 0, 10, 25, 50, 75 and 100 µg/ml were prepared using 5% dimethyl sulfoxide (DMSO: C_2H_6OS). Mushroom fraction/compound (0, 100, 250, 500, 750 and 1000 µl) of 100 µg/ml concentration (i.e., 0, 10, 25, 50, 75 and 100 µg/ml) and 500 µl of 20 mM sodium phosphate (Na_3PO_4) buffer (pH 6.8) containing 20 µl of standard active amylase (0.5 mg/ml) were incubated at 25°C for 10 min. Following pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated for 15 minutes. The reaction was halted by adding 1.0 ml of dinitrosalicylic acid, after which the tubes were placed in a boiling water bath for 5 minutes and then allowed to cool to room temperature. The reaction mixture was diluted with 10 ml of distilled water, and absorbance was measured at 540 nm. The standard control used was acarbose. The percent inhibition of amylase activity was calculated using the following formula (Eq. 8):

$$Inhibition(\%) = \left(\frac{Abs_{control} - Abs_{compound}}{Abs_{control}}\right) x\ 100 \ldots\ldots\ldots Eq.\ (8)$$

Inhibition Assay for β -Glucosidase Activity: As proposed by Elya, β- Glucosidase inhibition assay was performed (Elya et al., 2012). Phosphate buffer (100 mM, pH 6.8; 1 ml) and 0, 100, 250, 500, 750 and 1000 µl of the mushroom (100 µg/mL) such that 0, 100, 250, 500, 750 and 1000 µg/ml concentrations were mixed with 20 µL of active β-glucosidase (0.01 mg/ml) and incubated for 10 minutes in dark at 37 °C. To initiate the reaction, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG: $C_{12}H_{15}NO_8$) was added to the mixture. The reaction mixture was then incubated at 37°C for 60 minutes and terminated by adding 2.5 ml of 0.1 M Na_2CO_3. The β-glucosidase activity was assessed by measuring the absorbance at 400 nm. Control samples, prepared without any mushroom extracts, fractions, or compounds, were used for comparison, and acarbose served as the standard control. The % inhibition of β-glucosidase activity was calculated using Equation (Eq. 9).

$$Inhibition(\%) = \left(\frac{Abs_{control} - Abs_{compound}}{Abs_{control}}\right) x\ 100 \ldots\ldots\ldots\ldots\ldots Eq.\ (9)$$

Result and discussion:

Antioxidant Properties:

DPPH activity: *Pleurotus ostreutus* and *Lentinus sajor-caju* show increased DPPH activity with higher extract concentrations. Similarly, selected mushrooms exhibit rising DPPH activity with higher extract concentrations. The Graph1 presents DPPH radical scavenging activity of various mushroom species at different extract concentrations. The data highlights varied levels of DPPH radical scavenging activity among the mushrooms, suggesting their potential as antioxidant sources, with some species showing comparable or superior activity to ascorbic acid.

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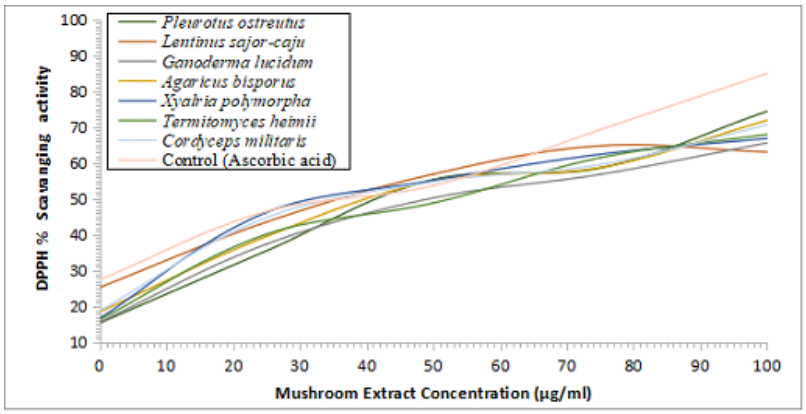
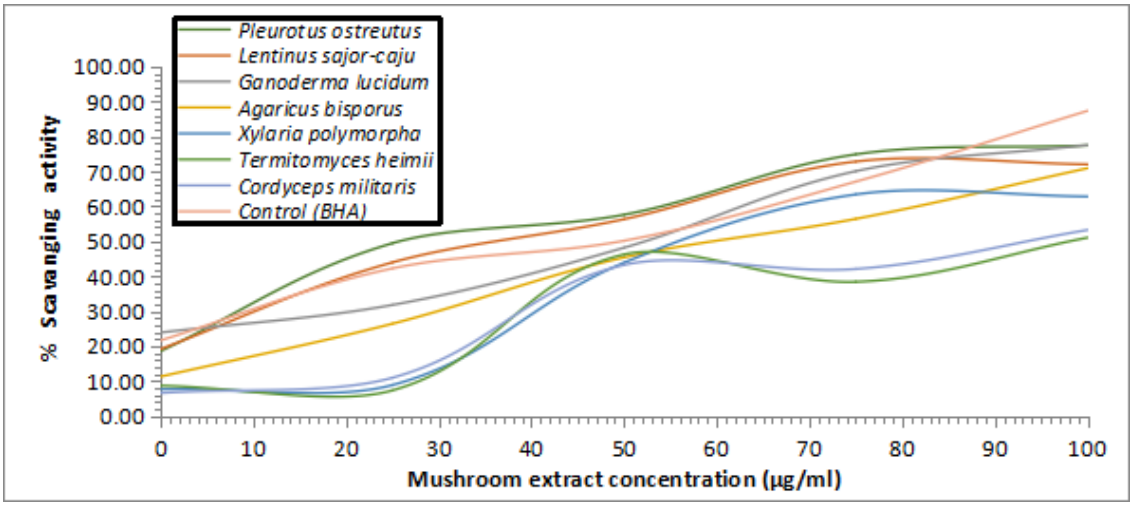


Graph1: *In-vitro* Antioxidant activity by DPPH method

H_2O_2 Superoxide radical scavenging activity*: Pleurotus ostreutus* and *Lentinus sajor-caju* exhibits pronounced radical scavenging activity. *Ganoderma lucidum* and *Agaricus bisporus* manifests potent radical scavenging capabilities., evident in consistently low IC_{50} values across the concentration spectrum. *Xylaria polymorpha* and *Termitomyces heimii's* radical scavenging activity presents a nuanced picture, with a decline observed at higher concentrations. *Cordyceps militaris* evinces more radical scavenging prowess with increasing concentrations. The control BHA, consistently demonstrates vigorous radical scavenging across concentrations. Notably, certain specimens, including *Xylaria polymorpha* and *Ganoderma lucidum*, exhibit comparable or superior antioxidant efficacy to the established control (BHA).



Graph 2: H_2O_2 Superoxide radical scavenging activity

NO reducing activity*:* Nitric oxide, a key signaling molecule plays a crucial role in various biological processes. The observed NO-reducing activity of these mushroom extracts suggests their potential relevance in modulating nitric oxide-related pathways, which could have implications for health and disease. Graph 3, elucidates the impact of different concentrations of mushroom extracts on nitric oxide levels.

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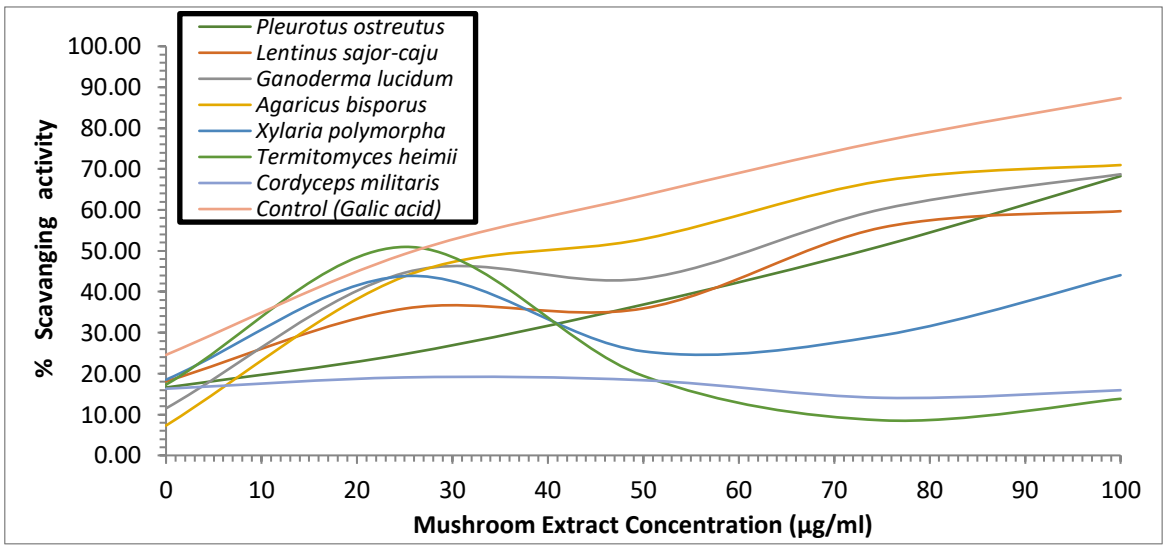
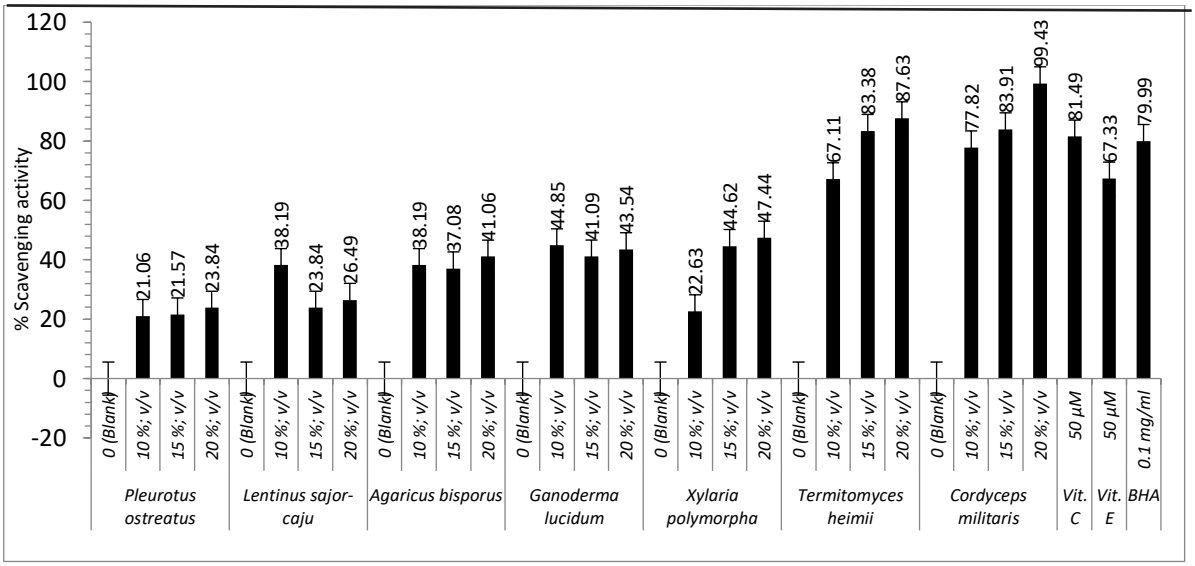


Graph 3: NO reducing activity

ABTS⁺ Scavenging capacity assay: Research has identified *Pleurotus ostreatus* as a source of antioxidants. *Lentinus sajor-caju* displays varying ABTS⁺ scavenging capacities at different concentrations. *Agaricus bisporus* and *Ganoderma lucidum* demonstrate signifying its efficacy in neutralizing ABTS⁺ radicals. The substantial increase in ABTS⁺ scavenging capacity with rising concentrations suggests potent radical neutralization by *Xylaria polymorpha* extracts. Specific information on *Termitomyces heimii* is limited, but the observed high scavenging capacity suggests the presence of potent antioxidants. The impressive concentration-dependent rise in ABTS⁺ scavenging capacity suggests potent antioxidant activity in *Cordyceps militaris* extracts.



Graph 4: ABTS⁺ Scavenging capacity assay

Reducing Power assay: The results, as presented provide insights into the reducing power of the mushroom extracts at different concentrations. The reducing power (Graph 5) decreases with increasing concentrations. Studies on *Pleurotus ostreatus* have shown its antioxidant potential attributed to phenolic compounds

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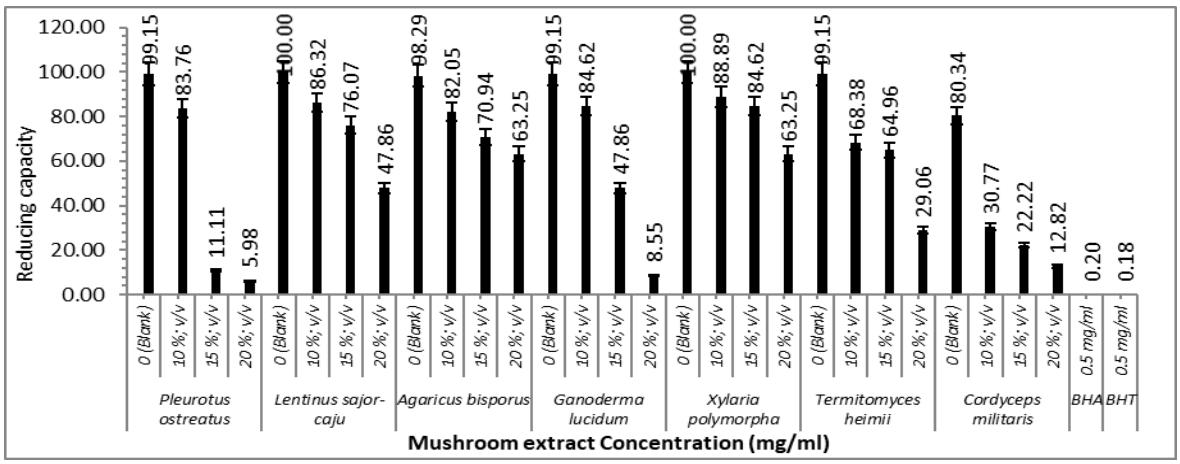
and flavonoids (Jayakumar et al., 2009). Similar to *Pleurotus* ostreatus, *Lentinus sajor-caju* exhibits a decreasing trend in reducing power as concentrations increase with evidence of phenolic compounds contributing to its reducing capacity (Sana et al., 2018).

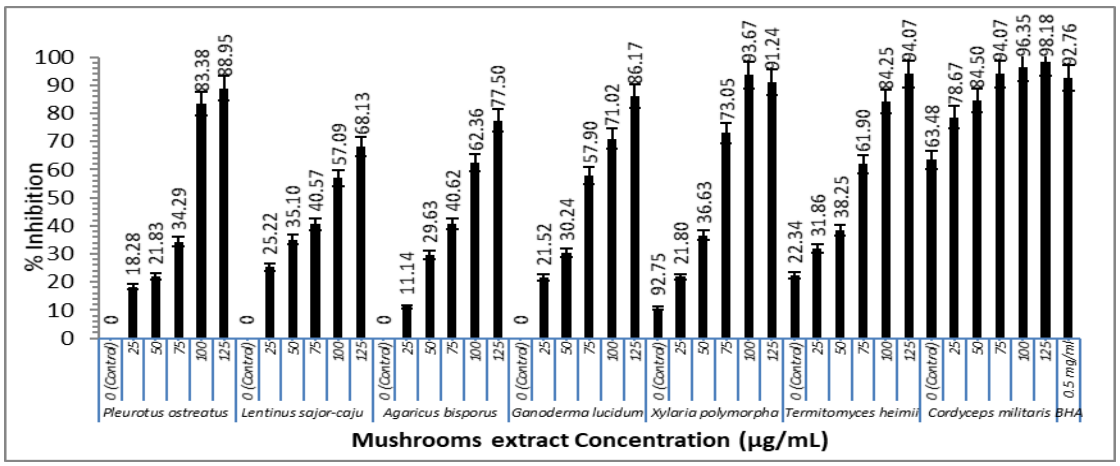
Research on *Agaricus bisporus* supports its potential as an antioxidant, with the presence of bioactive compounds contributing to its reducing capacity (Jeong et al., 2010).



Graph 5: Reducing Power assay

Ganoderma lucidum also exhibits a unique trend with an initial increase in reducing power at 10% v/v, followed by a decrease at higher concentrations. Studies on *Ganoderma lucidum* highlight its diverse bioactive compounds, including triterpenes and polysaccharides with antioxidant properties (Gao et al., 2003). *Xylaria polymorpha* shows a decrease in reducing power with increasing concentrations. *Termitomyces heimii* exhibits a decreasing trend in reducing power, aligning with higher concentrations leading to reduced absorbance. *Cordyceps militaris* is known for its antioxidant potential, attributed to cordycepin and polysaccharides (Wasser, 2011). BHA and BHT have established antioxidants with potent reducing capacities (Shahidi & Zhong, 2015).

Lipid Peroxidation Inhibition Assay: The Lipid Peroxidation Inhibition assay evaluates the ability to inhibit lipid peroxidation, a process associated with oxidative damage to cell membranes. The inhibition percentage increases with higher concentrations, indicating a concentration-dependent inhibition of lipid peroxidation.



Graph 6: Lipid Peroxidation inhibition assay

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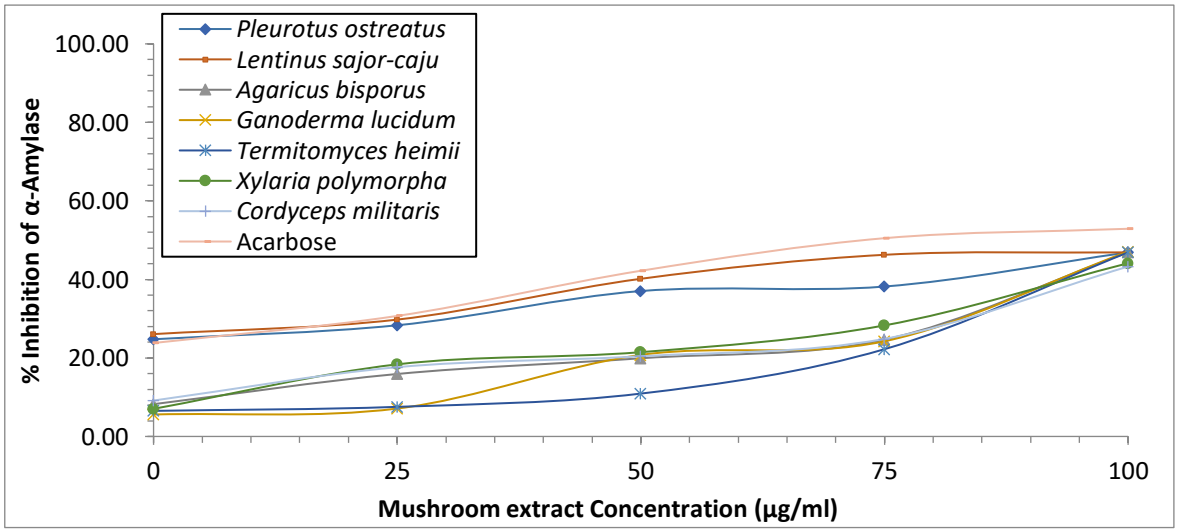
Pleurotus ostreatus has antioxidant properties attributed to its phenolic content (Jayakumar et al., 2009). Similar to *Pleurotus ostreatus*, *Lentinus sajor-caju* shows an increasing trend in inhibition percentage with rising concentrations. Studies on *Lentinus sajor-caju* have highlighted its antioxidant activities, potentially due to the presence of phenolic compounds. *Agaricus bisporus* shows a concentration-dependent increase in inhibition percentage, suggesting effective inhibition of lipid peroxidation. *Agaricus bisporus* has been recognized for its antioxidant potential, with compounds like ergothionein.(Sana et al., 2018). *Ganoderma lucidum* is well-known for its bioactive compounds, including triterpenoids and polysaccharides, which has antioxidant values (Gao et al., 2003). *Xylaria polymorpha* exhibits a substantial increase in inhibition percentage with rising concentrations, suggesting potent lipid peroxidation inhibition. *Termitomyces heimii* demonstrates a concentration-dependent increase in lipid peroxidation inhibition, emphasizing its potential as an antioxidant. While specific studies on *Termitomyces heimii* may be limited, the observed high inhibition percentage suggests the presence of potent antioxidants. *Cordyceps militaris* exhibits a concentration-dependent increase in inhibition percentage, indicating its effectiveness in inhibiting lipid peroxidation. *Cordyceps militaris* is recognized for its antioxidant content, including cordycepin and polysaccharides (Wang et al., 2012). BHA, a synthetic antioxidant control, shows a high inhibition percentage at 0.5 mg/ml. BHA is a well-known antioxidant, often used as a reference in studies. Its high inhibition percentage serves as a benchmark for the effectiveness of the mushroom extracts (Shahidi & Zhong, 2015). The observed concentration-dependent trends and high inhibition percentages are indicative of the potential health benefits of these mushrooms in combating oxidative stress and lipid peroxidation.

Antidiabetic properties:

α-Amylase % Inhibition assay: The α-amylase inhibition assesses the potential antidiabetic properties of various mushroom extracts by measuring their ability to inhibit the enzyme α-amylase.



Graph 7: α-Amylase % Inhibition assay

The percentage inhibition increases with higher concentrations, suggesting that *Pleurotus ostreatus* has been studied for its bioactive compounds, including polysaccharides and β-glucans, which may contribute to antidiabetic effects (Jayakumar et al., 2009). Similar to *Pleurotus ostreatus*, *Lentinus sajor-caju* exhibits a concentration-dependent increase in α-amylase inhibition. *Agaricus bisporus* has also been reported to contain bioactive compounds with anti-diabetic properties, including β-glucans and antioxidants (Jeong et al., 2010). *Ganoderma lucidum* exhibits a concentration-dependent increase in α-amylase

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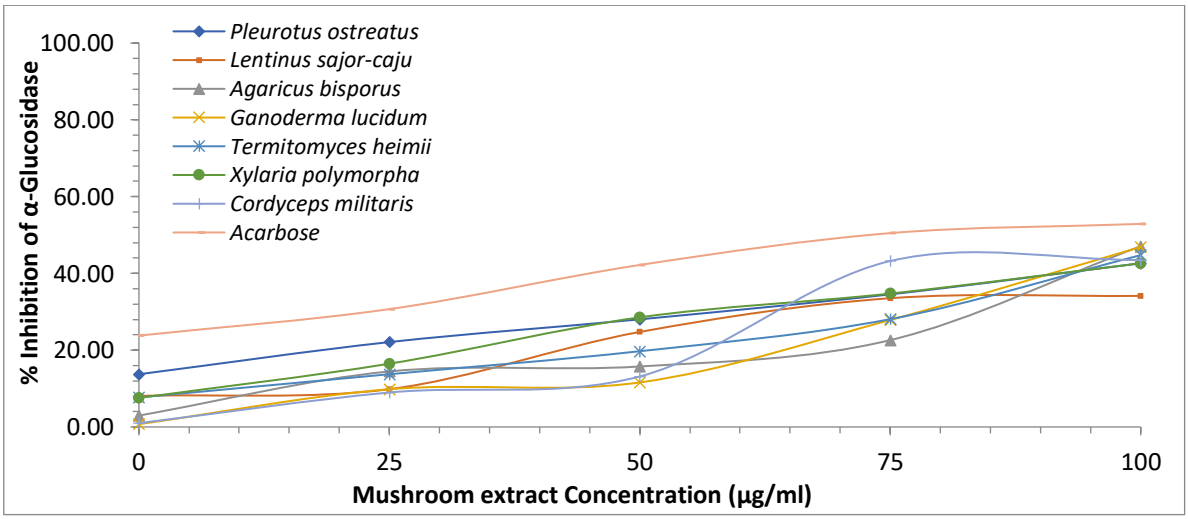
inhibition, and known for its diverse bioactive compounds, including triterpenoids and polysaccharides, which have been linked to antidiabetic effects (Gao et al., 2003). *Termitomyces heimii* demonstrates a dose dependent increase in α-amylase inhibition, suggesting potential antidiabetic activity. *Xylaria polymorpha* also exhibits a dose dependent increase in α-amylase inhibition, indicating its potential antidiabetic activity. *Cordyceps militaris* shows an increase in α-amylase inhibition, suggesting potential antidiabetic effects. Acarbose is a well-established α-amylase inhibitor used in the management of diabetes (Wang et al., 2012). The concentration-dependent increase in α-amylase inhibition aligns with the reported bioactive compounds in these mushrooms, emphasizing their potential as natural sources of antidiabetic agents.

β-Glucosidase % Inhibition assay*:* β-Glucosidase is involved in the digestion of carbohydrates, specifically in breaking down complex sugars into simpler forms like glucose. The substances tested include various mushroom extracts (*Pleurotus ostreatus, Lentinus sajor-caju, Agaricus bisporus, Ganoderma lucidum, Termitomyces heimii, Xylaria polymorpha, Cordyceps militaris*) and a reference substance Acarbose. Graph 8, presents the results of an β-glucosidase inhibition assay, which is a test used to evaluate the ability of different substances to inhibit the activity of the enzyme β-glucosidase.



Graph 8: β-Glucosidase % Inhibition assay

For example, at a concentration of 25 µg/ml, *Pleurotus ostreatus* exhibited a 22.11% inhibition of β-glucosidase activity. Similarly, at a concentration of 100 µg/ml, Acarbose showed a 52.9% inhibition. These results suggest the potential of the tested substances, particularly the mushroom extracts, to inhibit β-glucosidase activity. The higher the percentage of inhibition, the more effective the substance is in preventing the enzyme from breaking down carbohydrates into glucose. The reference substance, Acarbose, is included to provide a benchmark for comparison.

Conclusion:

In conclusion, our investigation into the antioxidant and antidiabetic properties of selected mushroom species from Gujarat has unveiled compelling evidence of their potential contributions to health and well-being. The antioxidant dimension, characterized by DPPH and H_2O_2 radical scavenging activities, NO-reducing activity, ABTS+ scavenging capacity, reducing power, and lipid peroxidation inhibition, showcases the diverse and potent antioxidant capabilities of these mushrooms. *Pleurotus ostreatus, Lentinus sajor-caju, Agaricus bisporus, Ganoderma lucidum, Xylaria polymorpha, Termitomyces heimii,* and *Cordyceps militaris* have demonstrated concentration-dependent antioxidant effects, rivaling or surpassing the efficacy of established antioxidants like ascorbic acid and BHA. In the realm of antidiabetic properties, the α-amylase % Inhibition and β-glucosidase inhibition assays provide valuable

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insights. *Pleurotus ostreatus, Lentinus sajor-caju, Agaricus bisporus, Ganoderma lucidum, Termitomyces heimii, Xylaria polymorpha,* and *Cordyceps militaris* exhibit concentration-dependent inhibition of α-amylase, suggesting their potential as natural agents in managing diabetes. Furthermore, the β-glucosidase inhibition assay reveals promising antidiabetic potential, with these mushroom extracts displaying significant inhibitory effects on the enzyme, similar to the reference drug acarbose. In essence, the multifaceted health attributes of these selected mushroom species open avenues for future research, emphasizing the need for a vigilant eye on evolving scientific literature. The intricacy of antioxidants and antidiabetic compounds within these mushrooms indicates exploration for their potential in boosting health and disease prevention, contributing to the dynamic landscape of mycological contributions to overall well-being.

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