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Antioxidant and anti-inflammatory activity of *Pleurotus citrinopileatus* Singer and *Pleurotus sajor-caju* (Fr.) Singer

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ABSTRACT

The antioxidant and anti-inflammatory properties of cultured mycelia from two species of the oyster mushroom, specifically *Pleurotus* species including *Pleurotus citrinopileatus* and *P. sajor caju* were investigated. The mushroom extracts were evaluated for their capacity to scavenge DPPH, hydrogen peroxide and free radicals. The antioxidant and anti-inflammatory activity was assessed through various assays, specifically the reducing power assay, hydrogen peroxide scavenging (H_2O_2) assay, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which exhibited similar activities across different concentrations of 100, 200, 300, 400 and 500 µg/ml. These diverse antioxidant activities of edible mushrooms compared to standard ascorbic acid were evaluated using a spectrophotometric method. The maximum percentage of antioxidant activities was observed in *P. citrinopileatus* and *P. sajor caju* at a concentration of 500 µl in DPPH assay when compared with low concentration as well as other methods in *P. citrinopileatus* mushroom and Vitamin C as standard. The effect of anti-inflammatory properties of aqueous extract of *P. citrinopileatus* with higher concentration of 500µl was good results when compared with methanolic extract and *P. sajor caju* mushroom with diclofenac sodium as standard. Nevertheless, the efficacy of both edible mushrooms suggested their promising potential for antioxidant properties in vitro condition. The antioxidant and anti-inflammatory activity exhibited by edible mushrooms holds considerable significance, as this activity substantially enhances their nutraceutical characteristics, thereby increasing their overall nutritional values.

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INTRODUCTION

Edible mushrooms have played a crucial role in human culture since ancient times (Chang and Miles, 2008) and those possessing medicinal properties have a long-standing history in the treatment of various human ailments (Ergonul *et al.,* 2013). Besides their rich content of micro and macronutrients mushrooms also generate a diverse range of secondary metabolites which include biologically active compounds. Among the approximately 14,000 known species of mushrooms, around 700 mushrooms are recognized as pharmacologically active (Guillamon *et al.,* 2010).

Some of these mushroom species are consumed directly or utilized as dietary supplements and functional foods (Aida *et al.,* 2009). Given their exceptional value as both food and medicine, the term 'mushroom nutraceutical' has been coined to refer to these medicinal mushrooms (Patel and Goyal, 2012). The genus *Pleurotus* encompasses various types of edible mushrooms that decay wood. Throughout history, numerous species of *Pleurotus* have been utilized in medicinal practices (Alves *et al.,* 2012); for instance, they have been employed to enhance the strength of joints, tendons and muscles, promote cardiovascular health (Heleno *et al.,* 2010) and boost the immune system. Presently, there are 207 recognized species within the *Pleurotus* genus (Mattila *et al.,* 2001).

Oxidation plays a crucial role in the energy production necessary for all living organisms to sustain their biological functions (Block and Patterson, 1992). Natural antioxidants are the subject of extensive research due to their ability to safeguard organisms and cells from harm caused by oxidative stress which is regarded as a contributing factor to aging and degenerative diseases (Gillman *et al.,* 1995). Substances that can be classified as food or components of food, which offer medical or health advantages such as disease prevention and treatment, are known as nutraceuticals (Selima *et al.,* 2011). Due to their antioxidant characteristics, mushrooms have gained popularity as a functional food and as a potential source for the creation of drugs and nutraceuticals (Jayasuriya *et al.,* 2020).

Among the category of higher fungi, edible medicinal mushrooms have a rich historical background in both culinary applications and traditional medicine. These species are known to possess biologically active compounds that may offer numerous health benefits to humans (Jan *et al.,* 2022). The *Pleurotus* genus exemplifies medicinal mushrooms particularly as *Pleurotus citrinopileatus* and *P. sajor-caju* stands out as one of the most widely cultivated edible mushrooms (Galappaththi *et al.,* 2021). *Pleurotus* is among the most significant mushrooms cultivated for commercial purposes globally, owing to its nutritional benefits. Additionally, it exhibits medicinal properties and other advantageous effects that are utilized in a range of health-related applications (Ghafoor and Naizi, 2024).

Golden oyster mushrooms (*P. citrinopileatus*) are well-known for their abundant essential nutrients and bioactive compounds such as flavonoids and carotenoids which enhance their antioxidant properties (Nor-Aleesya *et al.,* 2023). The mushroom species is especially proficient in alleviating oxidative stress, rendering it beneficial in dietary practices aimed at lowering the risk of chronic illnesses including cardiovascular disease. Additionally, its nutritional profile offers a favorable balance of essential amino acids and minerals establishing it as an outstanding dietary supplement for diverse demographic groups (Alona *et al.,* 2025).

Inflammation serves as a protective mechanism in response to tissue damage, typically resolving once healing has taken place (Mycek *et al.,* 2001). This process can be initiated by a benign agent or through an autoimmune reaction, as observed in rheumatoid arthritis (Banukie *et al.,* 2020). Mushrooms are regarded as a highly valuable nutritional resource owing to their high content of carbohydrates, proteins, free amino acids, vitamins, and a diverse array of essential minerals and trace elements (Colak *et al.,* 2009). Furthermore, it has been noted that mushrooms are rich in bioactive metabolites including polysaccharides, polyphenols, ergosterol and volatile organic compounds which enhance their medicinal attributes (Kalac, 2013). These metabolites derived from mushrooms have been recognized as

potential anti-inflammatory agents (Wang and Marcone, 2011; He *et al.,* 2012; Elsayed *et al.,* 2014). *P. citrinopileatus*, classified within the subphylum Basidiomycota, is acknowledged as an economically significant mushroom possessing both edible and medicinal qualities (Hyeok *et al.,* 2025).

MATERIALS AND METHODS

Antioxidant assay

DPPH assay (Oyaizu, 1986)

The standard methodology was employed to conducted the DPPH assay using different extracts of *Pleurotus citrinopileatus and P. sajor caju* mushrooms. Individually, the test mixtures of these mushrooms were prepared with different concentrations. Subsequently, 0.5ml of DPPH solutions was added to the each tube containing the samples. This experiment was performed in triplicate and allowed for incubated at room temperature in a dark field for the duration of 30 minutes. Finally, the radical scavenging activity was measured at 517nm using a UV spectrophotometer.

% of scavenged (DPPH): [(AC-AS/AC)] × 100

The absorbance of the control, denoted as AC and the absorbance in the presence of an extract sample, denoted as AS.

Reducing power assay (Oyaizu, 1986)

The method employed for determining the reducing power of the solvents extract involved several steps were followed. Initially, a mixture was prepared with 1ml of the sample of *Pleurotus citrinopileatus and P. sajor caju* which contained a concentration range of 0.2-0.8 microgram with 1ml distilled water. The 2.5ml of phosphate buffer and potassium ferrocyanide (1%) were added. The resulting solution was then incubated at a temperature of 50°C with 20 minutes. After the incubation period, 2.5ml of 10% TCA was added to the solution. The mixture was then spun at 3000rpm. The clear liquid on top was collected and mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. The absorbance of this mixture was measured at a wavelength of 700 nm. The absorbance value, it was compared with that of the standard ascorbic acid. The following formula was used for the determination of antioxidant activity.

Increase in reducing power (%)= {(A test-A std)/(A std)}×100

Hydrogen peroxide assay (H_2O_2) (Ruch et al., 1989)

The standard method was employed for scavenging assay. In order to assess the hydrogen peroxide assay of mushroom extract. Ascorbic acid served as the standard. The experiment used hydrogen peroxide solutions at different concentrations, specifically 0.6 mL of a 40 mM concentration. The spectrophotometer was used and measured the percentage of radical scavenging inhibitions at a wavelength of 230nm. The determination of scavenging inhibition was calculated and formula was performed.

Percentage of scavenged (H_2O_2): [(AC-AS/AC)] ×100

In-vitro anti-inflammatory activity (Chandra et al., 2012)

Determination of anti-inflammatory activity of *P. citrinopileatus* and *P. sajor-caju* were performed with egg albumin denaturation by *in-vitro* method. The mushroom extracts was used in the experiment consisted *P. citrinopileatus* and *P. sajor-caju* at various concentrations ranging from 50 to 400µg in each mushroom. Additionally, a one percent water mixture of BSA was included with them. Diclofenac sodium was utilized as the standard for comparison. The sample extracts at 100, 200, 300, 400 and 500µg/mL, along with the standard solutions were incubated at 37°C for twenty minutes. Subsequently, the turbidity of the samples was measured using a UV spectrophotometer at a wavelength of 600nm after the samples were chilled. The percentage of inhibition was then calculated using a standard formula.

% of inhibition = {(control OD – test OD) / Control OD × 100

RESULTS AND DISCUSSION

The scavenging activity was depending upon the concentration increased in the samples. This indicates a dose-dependent efficiency of DPPH scavenging by the mycelium extract.

Table 1. Antioxidant activity of *Pleurotus* species with DPPH assay

Different concentration (µl)	Standard (Vitamin C)	% of activity			
		Pleurotus citrinopileatus		*P. sajor-caju*	
		Aqueous	Hydrated methanol	Aqueous	Hydrated methanol
100	87.42±0.03	86.12±0.06	84.19±0.11	85.22±0.16	84.49±0.15
200	89.98±0.11	87.68±0.19	86.82±0.23	86.86±0.44	84.18±0.51
300	91.74±0.06	90.26±0.18	88.86±0.19	88.42±0.21	86.70±0.33
400	93.56±0.13	92.89±0.09	90.67±0.07	90.07±0.16	89.27±0.27
500	97.20±0.07	95.35±0.18	92.36±0.41	92.62±0.11	90.81±0.09

The values are expressed in terms of (Mean ± Standard deviation)

Table 2. Antioxidant activity of *Pleurotus* species with reducing power assay

Different concentration (µl)	Standard (ascorbic acid)	% of activity			
		Pleurotus citrinopileatus		*P. sajor-caju*	
		Aqueous	Hydrated methanol	Aqueous	Hydrated methanol
100	75.51±0.14	72.26±0.08	70.01±0.47	70.11±0.08	69.76±0.17
200	78.92±0.09	75.75±0.44	72.91±0.09	73.70±0.09	71.89±0.19
300	81.58±0.19	79.16±0.08	76.48±0.16	75.05±0.45	73.56±0.31
400	84.75±0.00	82.87±0.32	79.17±0.04	77.62±0.17	75.81±0.05
500	89.78±0.16	86.54±0.15	82.79±0.21	81.09±0.03	79.11±0.57

The values are expressed in terms of (Mean ± Standard deviation)

Table 3. Antioxidant activity of *Pleurotus* species with hydrogen peroxide assay (H_2O_2)

Different concentration (µl)	Standard (ascorbic acid)	% of activity			
		Pleurotus citrinopileatus		*P. sajor-caju*	
		Aqueous	Hydrated methanol	Aqueous	Hydrated methanol
100	68.16±0.07	67.16±0.05	65.06±0.00	65.03±0.11	62.09±0.14
200	72.38±0.11	70.85±0.01	67.13±0.09	69.48±0.05	65.18±0.01
300	75.18±0.00	74.01±0.10	72.29±0.02	71.08±0.17	69.03±0.12
400	78.22±0.05	77.19±0.05	75.16±0.07	73.15±0.02	71.01±0.01
500	80.17±0.13	79.35±0.08	77.31±0.01	75.03±0.00	73.16±0.07

The values are expressed in terms of (Mean ± Standard deviation)

Comparable findings have been documented in earlier research. The results obtained have been similarly reported in prior studies. Dundar *et al.,* (2013) noted that the ethanolic extracts derived from the mycelia of P. *eryngii, P. ostreatus, P. florida* and *P. sajor-caju* exhibited DPPH radical scavenging activities of 68.01%, 71.29%, 61.97%, and 62.82% at a concentration of 10 mg/mL^{-1}, respectively. The antioxidant activity of the hot water mycelial extract from P. *salmoneo-stramineus, P. ostreatus, P. eryngii* and *P. citrinopileatus* was found to range between 22% and 75% at 10 $mg.mL^{-1}$ (Smith, 2014). Despite *P. eryngii* having a brief growth cycle and yielding only two harvests, it exhibited the lowest production rate (PR) due to its also having the lowest biological efficiency (BE). The biological efficiency of most strains assessed indicated potential yields, with the exception of *P. eryngii*. The BE observed for *P. citrinopileatus* in this research was comparable to the findings of Musieba *et al.,* (2012).

Antioxidant activity were investigated in three different types of hydrogen peroxide scavenging assay, DPPH and reducing power assay method in which two different solvents (aqueous and hydrated methanol) and two different mushrooms (*P. citrinopileatus* and *P. sajor-caju*). The five different concentrations were used in this activity such as 100, 200, 300, 400 and 500 µg/ml. The 500 µl concentration of two solvents obtained the highest values for all antioxidant characteristics, and the 100µl concentration of aqueous and hydrated methanol were recorded at lower values, respectively. The two solvents; the maximum antioxidant activity analyzed in hydrated methanol extracts in both mushrooms are better scavenging reaction observed in DPPH > reducing power assay > hydrogen peroxide scavenging assay. Ascorbic acid and Vitamin C as a standard were used for the comparison of mushroom extracts (Table 1, 2 and 3).

Table 4. Anti-inflammatory activity of *Pleurotus* species

Different concentration (µl)	Standard (Diclofenac sodium)	% of activity			
		Pleurotus citrinopileatus		*P. sajor-caju*	
		Aqueous	Hydrated methanol	Aqueous	Hydrated methanol
100	80.25±0.03	78.33±0.02	76.51±0.17	75.11±0.05	72.58±0.01
200	83.37±0.28	81.11±0.26	78.63±0.02	78.57±0.13	75.22±0.28
300	86.07±0.04	85.38±0.00	83.10±0.13	82.03±0.05	80.47±0.02
400	89.11±0.13	87.27±0.16	86.06±0.15	85.26±0.07	83.16±0.00
500	93.54±0.27	92.19±0.03	89.25±0.02	89.44±0.19	86.24±0.19

The values are expressed in terms of (Mean ± Standard deviation)

The inflammatory response observed in the carrageenan-induced paw oedema test exhibits a biphasic nature, characterized by a maintenance phase occurring between 2 to 3 hours. The initial phase of inflammation is primarily driven by histamine and serotonin, alongside an increase in prostaglandin synthesis at the site of tissue damage, lasting for up to 2 hours. In contrast, the delayed phase of inflammation is mediated by leukotrienes, phagocytic cells, polymorphonuclear cells, monocytes, macrophages, prostaglandins produced by tissue macrophages, oxygen free radicals and nitric oxide and is typically observed from 3 to 5 hours. Throughout the maintenance phase, kinin-like substances, particularly bradykinin, become the predominant factors (Jedinak *et al.,* 2011).

Anti-inflammatory activity examined in *P. citrinopileatus* and *P. sajor caju* of two solvents (aqueous and hydrated methanol) and five different concentrations (100, 200, 300, 400 and 500 µg/ml). The highest anti-inflammatory activity recorded in aqueous and hydrated methanol extract of *P. citrinopileatus* were recorded and Diclofenac sodium as a standard, respectively.

The higher concentration of the *P. citrinopileatus* of aqueous extract was excellent results found to be recognized with little beatles activity of standard (Table 4).

CONCLUSION

The current research demonstrates the notable antioxidant and anti-inflammatory activity of the aqueous and hydrated methanol extract derived from the mycelia of two distinct *Pleurotus* species such as *P. citrinopileatus* and *P. sajor caju*. Incorporating medicinal mushrooms, known for their antioxidant characteristics, into the human diet could be beneficial in aiding the body to mitigate oxidative damage. The antioxidant activity exhibited by these edible mushrooms holds considerable significance, as it substantially enhances their nutraceutical properties thereby increasing their nutritional value. The research indicated that the culinary mushroom *P. citrinopileatus* and *P. sajor caju* exhibits anti-inflammatory properties and may be regarded as a functional food with the capability to manage inflammation. Possible mechanisms underlying the anti-inflammatory effects of *P. citrinopileatus* and *P. sajor caju* include antihistamine and membrane stabilizing activities, the inhibition of cell migration to the inflammation site, and the suppression of less production. Currently, anti-inflammatory activity-guided fractionation of the aqueous extract (AE) of *P. citrinopileatus* and *P. sajor caju* was underway to identify potent anti-inflammatory compounds. We advocate for clinical studies to assess the potential health benefits of the oyster mushroom.

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