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RESEARCH PAPER

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Cultivation and nutritional analysis of *Pleurotus* sp. from different substrates

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ABSTRACT

The oyster mushroom ranks as the second most significant mushroom globally as well as in India in terms of production. The scientific name for the oyster mushroom is *Pleurotus*. The cultivation process is relatively straight forward; it requires attention to several essential factors such as moisture levels, adequate sterilization and layered spawning to ensure optimal mycelial development and fruiting. Unlike the cultivation of button mushrooms, there is no requirement to compost the substrate, which may include straw or other materials used for mushroom growth. This type of mushroom can thrive on a diverse array of substrates and under varying temperature conditions. Certain species flourish at temperatures below 20°C, while others perform best at temperatures exceeding 20°C. *Pleurotus* mushrooms possess a high concentration of proximate nutrients, water-soluble vitamins and essential minerals. Various solvents, including aqueous, methanol, ethanol and diethyl ether were employed to extract bioactive compounds from *Pleurotus* species. *Pleurotus* mushrooms cultivated on three different substrates exhibited enhanced growth and nutritional properties. Based on the nutritional assessment of the *Pleurotus* species were analyzed. It can be concluded that this food source has the potential to play a significant role in addressing the growing food demands and alleviating micronutrient deficiencies in various regions particularly in developing nations.

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INTRODUCTION

Pleurotus mushrooms are regarded as nutritious due to their high content of proteins, fiber, vitamins and minerals. They are enjoyed as a functional food, appreciated for their appealing taste and aroma as well as their nutritional and medicinal benefits. The *Pleurotus* species, commonly known as oyster mushrooms, hold significant commercial value and are cultivated worldwide (Raman *et al.,* 2020). Approximately 998 million tons of agricultural waste are generated worldwide each year, encompassing materials such as paddy, wheat, and cereal straws. *Pleurotus* mushrooms thrive on these agricultural by-products, making their cultivation a valuable method for recycling waste and addressing the nutritional deficiencies that are particularly significant in regions like India. Furthermore, the residual substrates can be repurposed as fertilizers, animal feed and for biogas production (Kakon *et al.,* 2012).

Numerous species of *Pleurotus* have been identified and brought to market. Nevertheless, many of these species remain unexamined regarding their nutraceutical and medicinal properties. They produce extracellular enzymes that facilitate the breakdown of surrounding organic materials to acquire nutrients (Jegadeesh *et al.,* 2018). *Pleurotus* species are commonly found thriving on the moist trunks of trees and on decomposing organic matter that is abundant in lignin and phenol-degrading enzymes. These species are cultivated extensively using various agricultural by-products and employing straightforward, cost-effective production methods (Effiong *et al.,* 2024). These factors lead to a heightened vulnerability, prevalence and mortality associated with both communicable and non-communicable diseases (Beyene, 2023). The ongoing surge in the global population is projected to reach 10 billion by the year 2050 (Ulian *et al.,* 2020). The situation has prompted the exploration of alternative food sources that are easily accessible, cost-effective and nutritionally dense. Functional foods are defined as nutrient-rich substances that provide extra health advantages when ingested (Banerjee, 2019).

Mushrooms represent a valuable food source rich in nutraceuticals and possessing outstanding organoleptic qualities. Despite the toxic characteristics of many mushroom varieties, which have limited their consumption, several species of *Pleurotus* are both edible and highly nutritious (Fasoranti *et al.,* 2019). They have the capacity to function as functional foods and significantly enhance the nutritional value of meals when utilized as food additives, owing to their substantial levels of proteins, carbohydrates, minerals, vitamins, antioxidants, phytochemicals and low fat content (Allam and Mohamed, 2023). Mushrooms are greatly valued for their distinctive flavor and significant nutritional benefits, as well as their wide variety of dietary supplements. Consequently, they are regarded as an excellent source of non-starchy carbohydrates, dietary fiber, proteins, amino acids, minerals and vitamins (Elkanah *et al.,* 2022). *Pleurotus* ranks 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 variety of health-related applications (Galappaththi *et al.,* 2021). Mushrooms have been valued and used as food or food flavoring material not only for their texture and flavor but also for their chemical and nutritional characteristics and medicinal properties, offering numerous health benefits (Melanouri *et al.,* 2025). It is essential to evaluate the nutritional value of *Pleurotus ostreatus* in order to confirm its nutrient composition and establish its status as a functional food. The aim of this study is to evaluate the cultivation, preparation and nutritional analysis like proximate, vitamins, minerals and bioactive composition of *Pleurotus* species to determine its suitability as a functional food.

MATERIALS AND METHODS

Strains of *Pleurotus* species

Pleurotus sajor-caju and *Pleurotus citrinopileatus* strain was obtained from Indian Biotrack Research Institute, Thanjavur. The cultures were preserved on 2 % malt extract agar slants at 4° C. Subculturing was done after every 15 days.

Spawn preparation

Sorghum grains were prepared in polythene packets. The grains were subjected to boiling in a water bath for a duration of 10 to 15 minutes, maintaining a ratio of 1:1 (Sorghum grains to water). Subsequently, the grains were combined with 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$. Following this, 250 grams of the sorghum mixture were placed into polythene bags measuring 200x300 mm and sterilized in an autoclave at 121ºC for 30 minutes. After the sterilization process, the bags were inoculated with actively growing mycelium of *Pleurotus sajor-caju* and *P. citrinopileatus* obtained from malt extract slants and incubated at a temperature of 27±2 ºC for 10 to 15 days in the absence of light, allowing the mycelium to completely colonize the grains (Garcha, 1994).

Experimental details

A study was carried out utilizing a Randomized Block Design with five replications. The treatments involved the use of substrates, specifically paddy straw, wheat straw, and soybean straw, both individually and in a 1:1 combination. Each replication consisted of ten polythene bags corresponding to a single treatment. The yield data and various quality parameters were analyzed statistically to determine the level of significance, adhering to the recommended procedures.

Cultivation of *Pleurotus* sp.

Paddy straw, coconut coir and sugarcane trash materials their combinations in 1:1 ratio were used as cultivation substrates following the method described earlier. The preparation of the substrate involved soaking it in water for a duration of 72 hours, after which excess moisture was removed by spreading it on an inclined surface. Subsequently, the substrate was placed into polyethylene bags at a weight of 1 kg per bag and underwent dry sterilization (Khan *et al.,* 2006).

Yield and biological efficiency

Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. The biological efficiency (B.E. – yield of mushroom per kg substrate on dry wt. basis) was calculated by following the formula given by (Chang *et al.,* 1981).

Sample preparation

Pleurotus sajor-caju and *Pleurotus citrinopileatus* was washed to remove impurities and wiped neatly with sterile cloth to remove water traces on its surface. The washed *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* was dried using hot-air in an oven at 55–65°C till completely dried. The dried *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* was then ground to powder using a blender and weighed. The resulting powder obtained was 2.5 kg which was cooled to room temperature and stored in air tight containers for further use (Roghini and Vijayalakshmi, 2018).

Proximate analysis

Analysis of moisture, protein, fat, crude fibre, total carbohydrates, ash of samples were done by standard methods (AOAC, 1995).

Vitamin estimation

Folic acid, thiamin (B1), riboflavin (B2), and niacin were estimated according to Kamman *et al.* (1980). Vitamin C was estimated by the 2,6- dichloro phenolindophenol titration method (AOAC, 1995). Vitamin A and E using the method described by Majesty *et al.* (2019).

Determination of mineral elements

The prepared samples of *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* were analyzed for Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Potassium (K) and Zinc (Zn) using spectrophotometric methods according to Afolabi *et al.* (2023) and AOAC (2019).

Analysis of bioactive compounds

Qualitative bioactive analysis

The bioactive compounds such as alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and quinones were analysed with the solvents of aqueous, ethanol, methanol and diethyl ether extracts using standard method (Harborne, 1973) were followed. General reactions in these analysis revealed the presence or absence of these compounds in the mushroom extracts.

Quantitative bioactive analysis

The bioactive compounds such as alkaloids, amino acids, coumarins, phenols, steroids, terpenoids and quinones were analysed using standard method (Harborne, 1973), flavonoids (Bohm and Mohammed, 1994), saponins (Obadone and Ochuko, 2001) and tannins (Van and Robinson, 1981) were followed. These analysis revealed the amount of these compounds in the mushroom extracts.

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviations.

RESULTS AND DISCUSSION

Pleurotus species cultivated on paddy straw, coconut coir and sugarcane trash substrates exhibit a significant capacity for growth and yield. Among the two examined species of *Pleurotus*, which are characterized by their fleshy fruit bodies as illustrated in (Table 1 & Fig 1, 2a, 2b, 2c), *Pleurotus citrinopileatus* demonstrates a lower yield compared to *Pleurotus sajor-caju*. Recent research has extensively explored various aspects of oyster mushroom cultivation. Notably, *Pleurotus sajor-caju* shows an increase in mycelial growth of up to 100%, irrespective of the substrate composition, when compared to *P. ostreatus* and *P. florida* (Olasupo *et al.,* 2019).

Numerous factors affect the nutritional profile of edible mushrooms, with substrate composition being a significant determinant, as emphasized by (Belewu, 2003). The nutritional characteristics differ among species, and this variation is also influenced by the substrates used. The proximate analysis of *Pleurotus citrinopileatus* and *P. sajor-caju* revealed the percentage content of carbohydrate, moisture, ash, lipid, crude fiber and crude protein. The results showed that the carbohydrate content was highest, followed by, crude protein, ash, crude fiber and moisture as the least (Table 2). The results indicate that the oyster mushroom species evaluated possess a high nutritional value for human consumption.

Table 1. Growth, yield and biological efficiency of *Pleurotus* species using agricultural waste substrates

Different species of *Pleurotus*	Substrate	Spawn run (days)	Pinhead formation (days)	Yield (g) per harvest (g/kg)			Total yield (g/kg)	Bio-conversion efficiency (%)
				I	II	III		
Pleurotus citrinopileatus	Paddy straw	25	28	115	107	65	287	28.7
	Coconut coir pith	23	25	109	102	54	265	26.5
	Sugarcane trash	28	30	96	100	46	242	24.2
P. sajor-caju	Paddy straw	17	22	155	128	98	381	38.1
	Coconut coir pith	24	28	100	104	58	262	26.2
	Sugarcane trash	27	31	98	100	53	251	25.1

Table 2. Proximate analysis of *Pleurotus* species

Proximate content	Quantity	
	Pleurotus citrinopileatus	*P. sajor-caju*
Ash (%)	7.23	8.61
Moisture (%)	9.94	9.54
Carbohydrate (µg/g)	10.04	19.70
Protein (µg/g)	9.71	8.11
Crude fat (µg/g)	0.86	1.10
Crude fibre (µg/g)	4.16	5.41

Protein is a critical nutrient, and its deficiency is a leading cause of mortality worldwide.

Overall, the high carbohydrate content of *Pleurotus* species implies its potential as a viable source of energy in the diet which further justifies its use in making weaning food formula, breakfast meals.

Vitamins are essential chemicals substances required for the essential functioning of the body. They are needed regularly through the diet due to their importance in the growth and functioning of the body systems (Awuchi *et al.,* 2020). Vitamin A is a vital nutrient needed for vision, immune functioning and gene expression. They are good enhancers of certain minerals like zinc and Iron. Vitamin C also called ascorbic acid is an antioxidant and a strong reducing agent. It increases the absorption of non-haem iron in the gastrointestinal tract. They are good enhancers of certain minerals like zinc and Iron (Aslam *et al.,* 2017). The vitamin B1, B2, A, C and E contents of *Pleurotus citrinopileatus* and *P. sajor-caju* reported in this study was higher than the values (Table 3). The rich vitamin B content of *Pleurotus* species enables it function in boosting the immune system, enhancing DNA synthesis and improve red blood cells production, making it very suitable to be consumed by pregnant women, menstruating females and anaemic patients.

Fig. 1. Spawn preparation

Fig. 2a. Collection of substrates

Fig. 2b. Mushroom bed preparation

Fig. 2c. Cultivation of *Pleurotus* sp.

Table 3. Vitamin analysis of *Pleurotus* species

Vitamins	Quantity (μg/g)	
	Pleurotus citrinopileatus	*P. sajor-caju*
Vitamin A	2.13	2.47
Vitamin B_1	1.14	1.41
Vitamin B_2	2.00	2.16
Vitamin B_3	0.47	0.51
Vitamin C	1.19	1.74
Vitamin E	1.33	2.18

Minerals represent the ash content that remains after complete combustion of dry mushrooms. They perform vital functions in the body and are highly required for the growth and proper functioning of the body (Nwozo and Effiong, 2019). In the present study, *Pleurotus citrinopileatus* and *P. sajor-caju* was found to contain significant amounts of Mg, Fe, Zn, Na and lower amounts of Cu, Mn, P, K (Table 4). As a result, oyster mushroom is a viable diet that could help third-world countries overcome protein energy malnutrition and mineral shortage. Although the protein level is smaller than that of eggs, meat, or fish, it is sufficient to be used as a substitute in the general public's diet. These oyster mushroom species contain low fat content and high in unsaturated fatty acids, making it a nutritious food for all types of people.

Table 4. Mineral analysis of *Pleurotus* species

Minerals	Quantity (μg/g)	
	Pleurotus citrinopileatus	*P. sajor-caju*
Calcium (Ca)	9.50	13.40
Copper (Cu)	3.16	3.44
Iron (Fe)	7.42	9.58
Magnesium (Mg)	7.01	8.80
Manganese (Mn)	2.20	2.30
Nitrogen (N)	3.63	4.08
Phosphorus (P)	0.80	0.86
Potassium (K)	1.14	1.23
Zinc (Zn)	6.20	7.50

The analysis of bioactive components in edible mushrooms, specifically *Pleurotus citrinopileatus* and *P. sajor-caju* revealed the presence of significant bioactive compounds, including alkaloids and phenols (Table 4). These bioactive compounds are recognized for their health-enhancing properties. Saponins, for instance, represent a large family of structurally related compounds that include steroid and terpenoid derivatives. They are associated with numerous medicinal benefits, particularly anti-inflammatory and antidiabetic effects (Hamzah *et al.,* 2014). Consequently, these mushrooms may serve as therapeutic agents for diabetes and inflammatory conditions. Terpenoids have also been documented to exhibit a broad spectrum of pharmacological benefits, notably anti-inflammatory effects.

Table 5. Qualitative bioactive components of *Pleurotus* species

Bioactive compounds	Inference							
	Pleurotus citrinopileatus				*P. sajor-caju*			
	A	E	M	H	A	E	M	H
Alkaloids	+	+	+	+	+	+	+	+
Coumarins	+	-	-	+	+	-	+	-
Flavonoids	+	+	+	-	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-
Phenol	+	+	+	+	+	+	+	+
Saponins	-	-	-	+	-	-	+	-
Steroids	-	+	+	+	+	+	+	+
Tannins	-	-	+	-	+	-	+	-
Terpenoids	-	-	+	+	-	-	+	+
Quinones	+	-	+	+	-	-	+	-

(+) Present, (-) Absent, A – Aqueous, E – Ethanol, M – Methanol, D – Diethyl ether

Table 6. Quantitative bioactive components of *Pleurotus citrinopileatus*

Bioactive compounds	Quantity (μg/g)			
	Aqueous	Ethanol	Methanol	Diethyl ether
Alkaloids	05.05±0.03	06.10±0.03	06.09±0.06	07.02±0.06
Coumarins	09.03±0.00	-	-	08.03±0.23
Flavonoids	06.10±0.07	06.69±0.00	08.49±0.09	-
Phenol	06.51±0.01	06.38±0.03	07.44±0.43	06.26±0.08
Saponins	-	-	-	05.36±0.03
Steroids	04.45±0.00	03.74±0.03	03.17±0.09	04.75±0.05
Tannins	-	-	05.05±0.18	-
Terpenoids	-	-	03.36±0.36	04.63±0.02
Quinones	07.47±0.54	-	06.26±0.15	06.12±0.17

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

Phenolic compounds act as antioxidants and possess various therapeutic properties, including anti-cancer and anti-inflammatory activities. It is used for immunotherapy when the immunity of the organism is low and there are frequent infections and allergies (Iwona *et al.,* 2018). Thus, these mushrooms can be utilized in the management of oxidative stress. Given that phenols and flavonoids have demonstrated diverse antioxidant capabilities, they are employed in the treatment of stress-related disorders (Table 5). Flavonoids have been isolated from numerous mushroom species and have shown efficacy against various chronic diseases, with *Pleurotus citrinopileatus* and *P. sajor-caju* bioactive profile affirming its potential. The therapeutic effectiveness of these mushrooms may be attributed to the presence of secondary metabolites (Pandimeena *et al.,* 2015) which are integral to the human diet and confer a range of health benefits (Table 6 & 7).

Table 7. Quantitative bioactive components of *P. sajor-caju*

Bioactive compounds	Quantity (µg/g)			
	Aqueous	Ethanol	Methanol	Diethyl ether
Alkaloids	05.54±0.02	05.19±0.23	05.04±0.11	05.25±0.09
Coumarins	07.15±0.21	-	07.06±0.45	-
Flavonoids	06.02±0.09	06.45±0.80	06.35±0.12	05.48±0.21
Phenol	09.56±0.25	07.35±0.12	07.26±0.14	06.16±0.10
Saponins	-	-	02.08±0.16	-
Steroids	03.61±0.54	02.46±0.13	03.08±0.38	03.23±0.26
Tannins	07.06±0.67	-	08.42±0.06	-
Terpenoids	-	-	03.06±0.61	03.12±0.44
Quinones	-	-	06.11±0.34	-

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

CONCLUSION

This study evaluated the proximate, mineral, vitamins contents and bioactive compounds of *Pleurotus citrinopileatus* and *P. sajor-caju*. The proximate analysis results showed that *Pleurotus* sp. contained high carbohydrate and moisture contents (fresh sample of *Pleurotus* sp.), moderate protein and fiber content with low fat, ash and moisture contents (dry sample of *Pleurotus* sp.). Vitamins A, E and B2 were found to be present in high amounts in the *Pleurotus* sp. whereas other vitamins such as B1, B3 and C were found to be present much lower concentrations. The mineral content analysis revealed a high amount of calcium, iron and magnesium compared to other minerals. The maximum bioactive compounds were presented in the aqueous and methanol extracts of *Pleurotus* sp. The findings from these studies indicated that incorporating mushrooms into food products enhances both their nutritional content and physical attributes. Consequently, it is not unexpected that the food and pharmaceutical industries utilize mushrooms or their bioactive compounds to develop functional foods with nutraceutical benefits. By establishing a rapid, nutrient-dense food source, mushroom cultivation can provide individuals with a reliable income stream, thereby reducing their susceptibility to poverty and improving their overall quality of life.Overall, *Pleurotus* sp. was found to be rich sources of nutrients, vitamins, minerals and amino acids qualifying it to be a functional food and a valuable asset in the diet to help curb the rising incidence of nutrition related diseases.

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