RESEARCH ARTICLE

Phytochemical Profiling of *Pleurotus Pulmonarius* from Paddy Straw Substrate

Aswathy S, Manjunathan J*

Department of Biotechnology; School of life science; Vels Institute of Science Technology and Advanced Studies; Pallavaram; Chennai, Tamil Nadu, India.

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ABSTRACT

This paper focuses on the cultivation of *Pleurotus pulmonarius* mushroom, to find the Preliminary phytochemical analysis from five different solvent extracts of *P. pulmonarius*. Mushrooms are white fungi that are recognized as a good food source and contain a variety of medicinal benefits. In terms of nutrition, mushrooms are better than a lot of veggies because it contain around 40 to 49% protein. The current study integrates phytochemical analysis with the production of *P. pulmonarius* mushroom. The mushroom grew on paddy straw, which had been used as a substrate. The *Pleurotus* mushroom accounts for 25% of the total edible mushroom production worldwide. Fresh mushroom fruit bodies were picked, dried, pulverized, and extracted using a soxhlet apparatus. The pH, humidity and temperature of the surroundings were maintained in between the mycelia growth. Preliminary phytochemical analysis of five different extracts from *P. pulmonarius* was tested using various solvents, such as alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, saponins, proteins, quinones, carbohydrates and phenols. Further research can be conducted to determine the bioactive substances from this particular mushroom.

Keywords: Mushroom, *Pleurotus pulmonarius*, Oyster mushroom, Phytochemical, Substrate.

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INTRODUCTION

The most well-known name for *Pleurotus pulmonarius* is the grey oyster mushroom, although the Phoenix mushroom, the lung oyster, or the Indian abalone mushrooms. A greyish sporophore and a meat texture with a redolent character distinguish this. It is exceptional flavor and distinct fragrance makes it stellar esteem as a delicious fungus that has been utilized for decades as food and a substance that promotes food flavoring substance. In addition, it has a wide variety of polysaccharides, necessary amino acids, proteins, multivitamins, dietary fiber, and acids, and has a high sodium-to-potassium ratio but has low energy and fat contents.[1] According to Moradali *et al*. (2007), mushrooms are classified into two phylum, phylum Basidiomycota and phylum Ascomycota, they are known as higher fungi.[2] To increase food output and make additional revenue, traditional mushroom producers cultivate edible mushrooms with sawdust, cotton seed hulls and rice straw. In the 18th century, edible mushrooms were first grown for commercial purposes in France.[3] India produces about 160 million tonnes of cereals annually, and farmers also produce an equivalent amount of straw, certain quantities of which can be used for growing mushrooms. A wide variety of agricultural residue resources are readily available as substrates, including different types of wheat straw, corn cobs, straw of paddy, soybean, peanut and sugarcane bagasse. Substrates are essential because they function in the nutritional makeup of oyster mushrooms and earlier research has discovered astonishing differences in *Pleurotus* mushroom's proximate and mineral composition when grown in various substrates.[4] There are about 5000 species and 230 genera in the mushroom family. Of these, over 2000 species have been reported to be edible worldwide, around 283 of them reportedly being found in India. The pharmacological qualities of *P. pulmonarius* are vast, which include antioxidant, anti-cholinesterase, antitumor, antibacterial, immune-modulating and anti-inflammatory characteristics, and it may lower blood sugar levels also.[5] Many natural antibiotic sources have been widely utilized in recent years to treat a variety of illnesses that are infectious, mostly bacterial and fungal. Because of this, it is even more critical to find novel antimicrobial agents derived from medicinal plants and microorganisms in nations like India, where bacterial infections are not only common but also increasingly resistant to many commonly used antibiotics.[6,7] Because of the unfavorable toxicity of current

**Author for Correspondence: jmanjunathan@gmail.com*

medications and the rise in antibiotic-resistant strains caused by their careless and inappropriate use, the hunt for novel antibiotics is still ongoing.[8,9] Many plants and other natural substances are being used in the search for new antibiotics. These properties have been confirmed by numerous studies in the last few decades.[10-16]

The presence of phytochemicals in human diets has several beneficial health advantages. According to a study, mushrooms are a great source of beneficial medicinal compounds.[17] *Pleurotus* mushrooms have rich nutrients, phytochemicals, and a distinct flavor. Phytochemicals are naturally occurring substances that either directly prevent disease or act as a means of preventing disease in combination with nutrients.[18] According to study, oyster mushrooms are believed to be rich in high concentrations of phenolic, alkaloids, and flavonoids and to possess anti-helminthic activity.[19] Although more than sixty antimicrobial compounds have already been recognized.

The study reports and evaluates the phytochemical screening of five mushroom extracts, which are, ethyl acetate, acetone, hexane, chloroform and methanol. There is also the presence of saponins, terpenoids, steroids, glycosides, alkaloids and proteins, but the absence of tannins, phenols, carbohydrates, flavonoids and quinones. The current work analyzes the numerous bioactive compounds found in *P. pulmonarius* and subsequently determines its antimicrobial activity in order to illustrate *P. pulmonarius* potential as a safe and effective antibacterial agent.

MATERIALS AND METHODS

The sample of *P. pulmonarious* was collected from the Ranni forest division in the Pathanamthitta district, Kerala. It was then gathered it in a clean polyethylene bag. Taxonomic keys and descriptions were used to confirm the identification. Basidiomycetes were described based on their cultural, micro and macro characteristics using standard techniques. Thereafter, identified and compared its pigmentation, its morphology, the structure of hypha, size of the spore, and the spore-bearing structures of colonies. The sterilized fresh mushroom was taken for the culture of the fungi.

Step 1: Spawn development

P. pulmonarius spawn was produced by washing and soaking sorghum grains in water overnight. In an autoclave, the grains were boiled in water for 15 to 20 minutes. Then, drain the water from the grains and spread it on a clean mat. The grains were then mixed with $CaCO3$ (4%) and $CaSO4$ (2%) to optimize its pH and to avoid grain clumping.[20] The drained grains were then kept into clean bottles and before being sterilized at 121°C for around 30 minutes, ensure that it should be tightly sealed with aluminum foil and allow it to cool for a while, before being infused with rapidly spreading mycelia of *P. pulmonarius*.[21]

Furthermore, prepared sorghum grains were aseptically inoculated with the *P. pulmonarius* strains into different bottles. The grains that were inoculated were kept in a dark room at a temperature of 27 ± 2°C until the mycelial spawn run was reached, which happens when the grains were completely colonized.[22] (Figure 1).

Figure 1: Mother Spawn preparation

Step 2: Bed preparation and development of fruiting body

Paddy straw cut into long pieces (4–5 cm) and soak in water for around 2 to 4 hours and rinse it thoroughly.[23] After that, boiled it for sterilization *for* around 90 minutes. Then drain off the excess water, and the sterilized paddy straw placed on a wire mesh net and allow it to cool. It was then arranged into 12x24 cm bags (polypropylene) (Figure 2:A). Then, the sterilized spawns were scattered in the straw bed. After that the bags were punched with small holes for aeration. It was then placed in a dark room and kept the moisture level constant for 22-25 days. The cultivated wild mushrooms were harvested after 28 days of incubation (Figure 2 B, C, D & E, F).

Biological Efficiency

The formula for calculating biological efficiency

$$\text{Biological efficiency (\%)} = \frac{\textit{Total weight of the mushroom}}{\textit{The dry weight of the substrate}} \times 100$$

Preparation of Extracts for Phytochemical Screening

In order to stop fungus from growing, the newly harvested mushrooms were first cleaned and dried in sunlight for two to

Figure 2A: Bed preparation

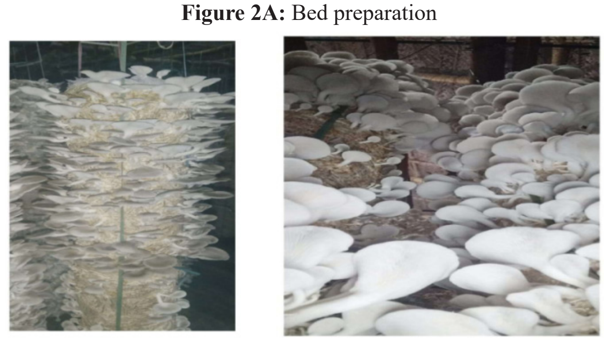

Figure 2: B and 2C: Fruiting body developmentt

Fig 2: D Harvested mushrrom

Figure 2E: Harvested mushroom

Figure 2F: Harvested mushroom

four days. They turned brown during this process then cut it into small, thereafter entire dried mushroom (Pileus and Stipe) was stored in a tight container. Using a household blender, grind the entire dried mushroom into powder form.

The powdered mushroom was extracted using a soxhlet apparatus. The solvents used here are methanol, chloroform, hexane, ethyl acetate, and acetone. The extracted substances were subsequently concentrated using a rotary evaporator and kept in a sealed container at 40°C till needed.

Preliminary qualitative phytochemical screening of *P. pulmonarius*

To generate an exact profile of the chemical composition of a given extract, a variety of qualitative chemical tests need to be performed.[24] It was then tested qualitatively for alkaloids; flavonoids; terpenoids; anthraquinones; saponins; carbohydrates; reducing sugars; tannins and phenolic compounds; cardiac glycosides; volatile oils and steroids .[24]

Saponin test

The resulting mixture of 2 mL of distilled water and 2 mL of water-based extract was shaken for a duration of 5 to 10 minutes. Saponin can be detected when a consistent layer of foam forms after 10 minutes.

Tannin test (Braymer's test)

5% alcoholic ferric chloride solution treated with 2 mL of extract. The dark blue color formation indicates tannin.

Terpenoid test (Salkowki's test)

Add chloroform (2 mL) and concentrated H_2SO_4 (few drops) in extract (2 mg). Its presence is immediately noticeable as a reddish-brown precipitate.

Phenol test (Ferric chloride test):

A 5% aqueous ferric chloride was treated with the extract, resulting in a deep blue or black color, which suggests the presence of phenol.

Steroid test (Liebermann buchar test)

Mix a few milligrams of extract with 2 mL of chloroform in a dry test tube. Heat the mixture after adding two to three drops of acetic acid, a few drops of acetic anhydride, and two drops of concentrated H_2SO_4. The formation of a green color reveals steroid.

Quinones test

Add a few drops of conc. H_2SO_4 to a few milligrams of extract. The appearance of red indicates the existence of quinone.

Glycosides test

The extract was treated with anthrone and conc. H_2SO_4. The presence of glycosides can be detected by the green color while heated in a water bath.

Carbohydrate test

Molisch's Test: Add one or two drops of alpha-naphthol and 2 to 3 mL of concentrated H_2SO_4 to the extract. Carbohydrates are indicated by a reddish-violet or purple ring at the interface of two liquids.

Fehling's test

The extract (1-mL) was boiled in a water bath, and Fehling solutions A (1-mL) and B (1-mL) were added to it. A red precipitate shows the existence of sugar. For the preparation of Fehling s solution A, dissolving $CuSO_4$ (34.66 g) in distilled H_2O and diluting it to 500 mL. Fehling's solution B has been made by dissolving potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}_4H_20$) (173 g) and NaOH (50 g) in water (500 mL).[25]

Benedict's test

CuSO4 (17.3 g) dissolved in 100 mL of distilled water was added after Benedict's reagent (Sodium citrate (173 g) and Na2CO3 (100 g) dissipated in 800 mL of distilled water and boiled to form a clear solution). In a boiling water bath, combine 1-mL of Benedict's reagent with 0.5 mL of extract and heat for 2 minutes. A precipitate with a distinct color indicated the presence of sugar.[25]

Alkaloid test

- *Dragendorff's test*

Just a few milligrams of the substance/extract were heated for two minutes in a test tube containing 2% H2SO4 before being filtered in the same test tube. After that, a small amount of Dragendorff's reagent-bismuth carbonate (5.2 g) along with sodium iodide (4 g) was heated to a boil in 50 mL of glacial acetic acid for a short while. After 12 hours, a reddish-brown filtrate of 40 mL of clear solution combined with 60 mL of ethyl acetate and 1-mL of water was obtained, which was then placed to an amber-colored container. The precipitated sodium acetate crystals were filtered through a sintered glass funnel. The presence of alkaloids is indicated by the orange-red precipitates.[25]

Flavonoid test

Add 10% NaOH or ammonia into the extract in alcohol. The appearance of a dark yellow color can detect flavonoids.

Protein test (Biuret Test)

Add a small amount of very diluted (1%) copper II sulphate solution to the sample solution after adding sodium hydroxide solution. Mix gently. The purple appearance indicates the presence of protein.

RESULTS AND DISCUSSION

The findings in the study included mushroom yield, identification and phytochemical analysis (Table 1).

Mushroom *P. pulmonarius* was grown at a room temperature of 26°C. For humidity, water was sprayed on paddy straw and maintained at 80 to 85% constantly. This species needs high humidity to develop properly. The growth was observed in raw materials in 17 to 25 days and the first harvest can be made.

Identification

For identification purposes, standard methods and manuals are used, like the manual of soil fungi; identification is purely based on its macro, micro, and cultural characteristics. From its characteristics, it identified that the mushroom was *P. pulmonarius* and its GenBank accession number is CQ727110 (Figure 3).

Preliminary Phytochemical Analysis

The extracts of *P. pulmonarius* were found to contain protein compounds, glycosides, terpenoids, alkaloids, steroids, and saponins, according to a preliminary phytochemical screening (Table 2).

Table 1: Optimization condition of *P. pulmonarius*

Substrate	*Paddy straw*
Ph.	6.5
Temperature	25–30°C
Crop cycle	45–47 days
Humidity	80–85%
First harvest	18^{th} day
Yield per bag	1500 g

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Go to:

LOCUS       OQ727110                 545 bp    DNA     linear   PLN 08-APR-2023
DEFINITION  Pleurotus pulmonarius isolate 65345Vels internal transcribed spacer
            1, partial sequence; 5.8S ribosomal RNA gene, complete sequence;
            and internal transcribed spacer 2, partial sequence.
ACCESSION   OQ727110
VERSION     OQ727110.1
KEYWORDS    .
SOURCE      Pleurotus pulmonarius
  ORGANISM  Pleurotus pulmonarius
            Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
            Agaricomycetes; Agaricomycetidae; Agaricales; Pleurotineae;
            Pleurotaceae; Pleurotus.
REFERENCE   1  (bases 1 to 545)
  AUTHORS   Aswathy,S. and Manjunath,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-APR-2023) BIotechnology, Vels University, Pallavaram,
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ORIGIN
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      301 aatctttgaa cgcaccttgc gccccttggt attccgaggg gcatgcctgt ttgagtgtca
      361 ttaaattctc aaactcacat ttatttgtga tgtttggatt gttggggtt gctggctgta
      421 acaagtcggc tcctcttaaa tgcattagca ggacttctca ttgcctctgc gcatgatgtg
      481 ataattatca ctcatcaata gcacgcatga atagagtcca gctctctaat cgtccgcaag
      541 gacaa
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Figure 3: GenBank accession details of the sample

The extract of hexane contains alkaloids, terpenoids and steroids. On the other hand, the chloroform extract revealed only the presence of terpenoids and alkaloids. Certain substances, including alkaloids, steroids, and terpenoids, are mostly seen in ethyl acetate. Methanol extract shows the presence of compounds such as terpenoids, glycosides and alkaloids, but in acetone extract it indicates the presence of terpenoids, steroids, glycosides, alkaloids and proteins. Commonly, all five extracts contain alkaloids and terpenoids. But tannins, phenols, carbohydrates, flavonoids and quinines were absent.

Phytochemical analysis indicates that the mushroom is abundant in nearly every kind of secondary metabolite, which is required for everyday life due to these phytochemicals.[25] They not only play a normal physiological role but also strengthen interconnected biological systems.[26] Research has demonstrated that the secondary metabolites present in mushrooms offer a range of health advantages, including anti-inflammatory and antioxidant characteristics. Additionally, their potential for preventing chronic diseases and boosting the immune system has also been widely studied. Regular mushroom consumption can offer a safe, natural means of promoting overall health and well-being.[27-31] The diverse range of secondary metabolites found in mushrooms not only contribute to their antioxidant and anti-inflammatory properties, but they maintain a balanced immune system also.

Table 2: *P. pulmonarius* a qualitative phytochemical analysis using various organic solvents.[25]

Tests	*Extracts*				
	Hexane	*Chloroform*	*Ethyl acetate*	*Acetone*	*Methanol*
Saponins	+	-	-	-	-
Tannins	-	-	-	-	-
Terpenoids	+	+	+	+	+
Phenols	-	-	-	-	-
Steroids	-	-	+	+	-
Glycosides	-	-	-	+	+
Carbohydrates	-	-	-	-	-
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	-	-
Proteins	-	-	-	+	-
Quinones	-	-	-	-	-

Therefore, incorporating mushrooms into one's daily diet can be a valuable strategy for promoting long-term health and disease prevention.

CONCLUSION

The main outcome of this study is that paddy substrate produced a higher output of mushrooms in a cheaper and faster manner. With less than five days of growth cycles, the mushrooms grew quicker on the substrate. There are various edible mushrooms that have not been studied for their possible application in cuisine. These novel fungal species may have distinct biological processes, sensory organs, and environmental effects compared to previous species. As a result, these various species must be studied. Furthermore, much analysis is required to discover the mechanisms of action of the fungus's numerous components, such as its antibacterial activity, inhibitory activity, structure, textural features, and taste qualities. Mushroom benefits are relatively inexpensive because mushrooms are cultivated on a range of agricultural or forest wastes, such as rice straw, corncobs and sawdust. Antifungal inoculates can be generated in a factory using simple processes that do not normally produce fungus. When seeking cost-effective and environmentally friendly restoration options, mushrooms can be a wonderful approach.

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