IDENTIFICATION AND TISSUE CULTURING OF WILD MEDICINAL MUSHROOMS

Project submitted

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Submitted by

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DEPARTMENT OF BOTANY

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CERTIFICATE

This is to certify that this project work entitled "**IDENTIFICATION AND TISSUE CULTURING OF WILD MEDICINAL MUSHROOMS**" is a bonafide piece of project work done by Gouthamy Rajeev, AaryaVR, Rabina k Raveendran 21002022651,210021022641,210021022659 in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

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DECLARATION

I hereby declare that this project entitled "**IDENTIFICATION AND TISSUE CULTURING OF WILD MEDICINAL MUSHROOMS** " is the result of work carried out by me under the guidance of **Dr.Shahina NK,** Department of Botany,Bharata MataCollege, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

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CHAPTER 1

INTRODUCTION

Mushrooms are the fascinating fruiting bodies of fungi. They are mostly basidiomycetes but also some are ascomycetes. They are macrofungi, large enough for us to see and hold by hand. Over 140,000 species are reported though only 10% are currently identified. They can be fleshy, woody, or leathery, and reproduce by releasing spores from gills or tubes on their undersides.

Humans use mushrooms as a source of food and cultural significance. Early humans likely discovered edible varieties through trial and error, and civilizations from Greece to China have revered them for their health benefits, flavor, and even supposed magical properties.

Since ancient times, mushrooms have held a special status as food. Greeks attributed mushrooms to enhancing the strength of warriors in battle, while Pharaohs valued them as a delicacy, and Romans deemed them the "Food of the Gods," often serving them only during celebrations. In Chinese culture, mushrooms were cherished for their health benefits. Consumption of mushrooms likely dates back to the earliest human history. While historical records primarily focus on wild mushrooms, stories suggest that mushroom cultivation in Asia dates back to the early Middle Ages (Swapna et al., 2008)

1.1 Diversity of Mushrooms

Mushrooms exhibit diverse habitats, including soil, dead leaves, wood, and decomposing organic matter, each influencing their species diversity. They are classified based on their nutrition into saprophytes, parasites, and symbiotic organisms. Mushrooms predominantly thrive during the rainy season in forests, displaying various shapes, sizes, and colors. Environmental factors like temperature, humidity, light, and substrate composition profoundly impact their growth. Despite their diversity, mushrooms share common nutritional and ecological requirements, which categorize them into different ecological groups. Overall, mushrooms represent a fascinating aspect of biodiversity, showcasing remarkable morphological diversity and ecological adaptability (Kumar et al., 2011; Reddy, 2015).

1.3 Significance of mushrooms

Mushrooms are significant in ecological, nutritional and medicinal aspects.

***Ecological role*:** Mushroom habitat and climate are the major factors that indicate their biodiversity. They play an important role as decomposers by breaking down plant components like lignin and cellulose. In this process, they also degrade surface waste and contribute to nutrient cycling by releasing nitrogen into the soil as ammonium nitrate. This nutrient is essential for plant growth and survival, highlighting the significant ecological importance of mushrooms in ecosystem functioning.

***Health benefits* :** Mushrooms are considered as functional food renowned as a complete suitable for individuals of all ages, from children to the elderly. Their nutritional value is influenced by factors such as species, developmental stage, and environmental conditions. They are in protein, dietary fibers, vitamins, and minerals, mushrooms contain approximately

eighty to ninety percent water and eight to ten percent fiber. They are particularly abundant in vitamins C and B (including folic acid, thiamine, riboflavin, and niacin), as well as minerals like potassium, sodium, and phosphorous. They also contain trace amounts of essential minerals such as copper, zinc, and magnesium and many species are rich in iron and calcium (Eg: Chantarella, Termitomyces). Mushrooms have low lipid levels with a higher proportion of polyunsaturated fatty acids, resulting in a low-calorie yield. Furthermore, they do not contain cholesterol but rather ergosterol, which serves as a precursor for vitamin D synthesis in the human body. While the protein content of edible mushrooms is generally high, ranging from 12 to 35 percent depending on the species, their free amino acid composition varies widely, with a particular richness in threonine and valine but a deficiency in sulfur-containing amino acids (Shahina, 2019) (Shahina et al., 2022).

1.5 Life cycle of Mushrooms

The life cycle of a mushroom starts with spore germination, where tiny hyphae develop into mycelium under favorable conditions of moisture and nutrient availability. Mycelium, a network of thread-like structures, grows by breaking down organic matter and absorbing nutrients. Under specific environmental conditions, primordia or pins form from the mycelium, eventually developing into fruit body of fungus, mushrooms. The mushrooms continue to grow, with the cap expanding and the stem elongating, while the mycelium provides nutrients for their development. As the mushrooms mature, they produce spores from gills or pores underneath the cap. Once fully developed, the mushrooms are ready for spore dispersal, completing the cycle.

1.6 Mushrooms domestication and culturing

The intentional cultivation of wild mushrooms by humans from their natural habitat to controlled environments is known as mushroom domestication. The process starts with specific potential mushroom species, tissue/spore culturing under aseptic conditions in suitable media for pure culturing and further spawn production in suitable substrates. For ruit body production has to create optimal growing conditions, by managing temperature, humidity, substrate composition, and light exposure. Domestication allows for the consistent production of high-quality mushrooms, enabling their availability throughout the year and reducing reliance on wild harvesting. Additionally, domestication facilitates the development of improved cultivation techniques, genetic selection for desirable traits, and innovation in mushroom farming practices. Overall, mushroom domestication plays a crucial role in meeting the growing demand for mushrooms as a nutritious food source and functional ingredient in various industries (Manoharachary et al., 2016).

OBJECTIVE

- Collection and identification of diverse species of wild mushrooms from their natural habitats.
- Literature study of the medicinal properties associated with the identified mushroom species.
- Conduct tissue culture for the pure culturing of selected mushrooms
- Conduct qualitative phytochemical analysis of aqueous extracts of one potential mushroom

SIGNIFICANCE

The study Provides the information of ecology and the diversity of wild mushroom species. By stablishing tissue culture techniques for the controlled cultivation of mushrooms, enabling further research and applications. Identifying and characterizing bioactive compounds present in mushroom extracts may be used for further quantitative studies of the potential compounds. Review of the existing literature to consolidate knowledge on the medicinal properties of wild medicinal mushrooms,will aid in their utilization for healthcare and pharmaceutical purposes.

CHAPTER 2

REVIEW OF LITERATURE

Mushrooms exhibit remarkable diversity in terms of species, morphology, and ecological roles. They belong to the kingdom Fungi and play crucial roles in nutrient cycling, mycorrhizal associations, and decomposition processes in various ecosystems

The diversity of mushrooms extends from the microscopic to the macroscopic, encompassing various forms like molds, yeasts, and multicellular fruiting bodies. From the edible and medicinal mushrooms like Shiitake (*Lentinula edodes*) and Reishi (*Ganoderma lucidum*) to the highly toxic species such as the Death Cap (*Amanita phalloides*), the ecological roles and applications of mushrooms are diverse and multifaceted (Chang & Miles, 2004).

Research by Natarajan et al. (2005) and Brown et al. (2006) provided comprehensive insights into the ectomycorrhizal fungi diversity found in the Western Ghats of Karnataka. These studies not only cataloged the various species of ectomycorrhizal fungi present but also explored their ecological roles and interactions within the ecosystem. Ectomycorrhizal fungi form symbiotic relationships with the roots of plants, particularly trees, enhancing nutrient uptake for the host plants while receiving carbohydrates in return. This mutualistic association plays a vital role in the health and vitality of forest ecosystems. The research emphasized the importance of these fungi in maintaining soil fertility, promoting plant growth, and contributing to the overall biodiversity of the Western Ghats. Through their

detailed analyses, Natarajan et al. and Brown et al. highlighted the significance of understanding fungal diversity and their symbiotic relationships with plants for effective conservation and sustainable forest management in the Western Ghats region.

Swapna et al. (2008) conducted an extensive survey focusing on the macro fungi diversity in both semi-evergreen and moist deciduous forests of Karnataka. Their research employed rigorous field sampling techniques and laboratory analyses to identify and document the fungal species present. By examining various forest types, they were able to identify significant variations in fungal diversity, abundance, and distribution patterns. The study also explored the ecological preferences of different fungal species, shedding light on their habitat requirements and microenvironmental factors influencing their occurrence. Swapna et al.'s work not only provided a comprehensive inventory of macro fungi but also offered insights into the ecological roles these fungi play within their respective ecosystems. Furthermore, their findings underscored the importance of conserving diverse forest types to maintain fungal biodiversity and ecosystem health in the region.

Different forest ecosystems in Kerala supported a diverse assemblage of mushroom communities, influenced by factors like microclimate, physiological conditions, and human activities. Kerala's rich macro fungal diversity was particularly pronounced in its moist-deciduous and semi-evergreen forests, followed by evergreen and Shola forests. Studies reported over 166 genera and 550 species of mushrooms belonging to both Basidiomycota and Ascomycota across various forests in Kerala (Farook et al., 2013; Mohanan, 2014).

Thiribhuvanamala et al. (2011) conducted a detailed study on the seasonal occurrence of mushrooms in the Western Ghats. They meticulously documented the timing of mushroom

fruiting events and correlated these patterns with seasonal and climatic variations. By doing so, they provided valuable insights into the temporal dynamics of fungal life cycles in this biodiverse region. Their research highlighted the influence of environmental factors such as temperature, humidity, and rainfall on mushroom fruiting, emphasizing the intricate relationship between fungi and their surrounding ecosystem.

In another study focusing on urban mycology, Pushpa and Purushothama (2012) explored the abundance and diversity of microfungi in and around Bangalore. Utilizing diversity indices, they quantified the presence of various microfungi species and assessed their distribution across urban and peri-urban landscapes. Their systematic assessment revealed the resilience of fungal communities in adapting to urban environments and provided insights into the potential impact of urbanization on fungal diversity. Additionally, their findings highlighted the importance of preserving green spaces and natural habitats within urban areas to maintain fungal biodiversity and ecological balance.

Varghese et al. (2010) identified approximately 21 species of wild edible mushrooms that hold tribal importance in the Wayanad district of Kerala. This study highlighted the cultural and nutritional significance of these mushrooms to the local communities.

In Thiruvananthapuram District, Vrinda and Pradeep (2010) investigated the ectomycorrhizal fungal diversity across three distinct forest types. Their findings revealed a higher diversity of fungi in evergreen forests compared to exotic forests, emphasizing the ecological importance of native forest ecosystems.

Farook et al. (2013) compiled a literature-based checklist focusing on gilled mushrooms from Kerala. Their study suggested that many mushroom species in the region could be endemic, indicating the unique fungal diversity of Kerala.

Mohanan (2014) explored the macro fungal diversity within the Russulaceae family in Kerala's forests. The study highlighted their ectomycorrhizal associations with various tree species such as Hopea ponga, H. parviflora, Myristica malabarica, Vateria indica, and *Calophyllum apetalaum,* underscoring the intricate ecological relationships between fungi and trees in these forests.

Vrinda (2014) further expanded the knowledge on wild edible mushrooms in Kerala by collecting and identifying 85 species from the region's forests, contributing to our understanding of the rich fungal biodiversity and its utilization by local communities.

In the latest ethnomedicinal ecological studies, Shahina (2019) conducted research in Attappadi and Wayanad focusing on the traditional knowledge and ecological aspects of wild edible mushrooms. This study provided insights into the ethnomedicinal uses of mushrooms by local communities, their ecological roles, and conservation concerns related to these valuable fungal resources.

Lignicolous mushrooms, which grow on wood, have gained attention for their medicinal properties and ecological roles. In traditional medicine, these mushrooms have been used for liver protection and overall well-being, highlighting their therapeutic potential. The genus Auricularia includes species like Jude's Ear (*Auricularia auricula-judae*) and Wood Ear (*Auricularia polytricha*), which are known for their immune-boosting compounds. Additionally, mushrooms such as Turkey Tail (*Trametes versicolor*) and *Phellinus linteus* are rich in antioxidants and have shown potential in combating oxidative stress and supporting immune health. These mushrooms also exhibit anti-inflammatory and antimicrobial effects, making them promising for various health conditions. Reishi mushroom (*Ganoderma*

lucidum), another lignicolous species, is being studied for its potential anticancer properties and liver protective benefits (Wasser, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials Required

The study required certain materials and equipments

For mushroom collection:

Mushroom Identification Guides: Books or apps to help identify different mushroom species.

Collection Containers: Paper bags, Wax paper for spore prints

Knife or Small Shovel: For careful collection without damaging the mushroom or its mycelium.

Field Notebook and Pen: To record collection details like location, habitat, and date.

Camera: Helpful for documenting mushrooms in their natural habitat.

For Tissue Culture:

Sterile Petri dishes or culture vessels, Growth medium (e.g., agar-based or liquid), Sterile instruments (scalpels, forceps, pipettes), Sterilization equipment (autoclave, ethanoll, Incubator or growth chamber with controlled temperature and light conditions, Laminar flow hood or biosafety cabinet for sterile work, Chemicals for medium preparation (sugars, salts, vitamins,) Mushroom tissue, Pileus or stipe

3.2 Methods

3.2.1 Collection and storage of mushroom

The collection trial focused on gathering fully matured mushroom species across different stages of development to study a comprehensive range of characters. Each mushroom was carefully uprooted using a scalpel or sharp knife to preserve its integrity. Before collection, photographs were taken in their natural habitats to document the mushrooms in situ. Detailed data on habit, habitat, soil type, location, microhabitat, morphology, and color were meticulously recorded in the field. Every specimen was then placed in butter paper bags, assigned a unique collection number for identification purposes.

The collection sites were predominantly in the regions of Thattekad and Idukki, with an emphasis on diverse habitats such as decaying wood and forested areas. Special attention was given to note the host tree, color, shape, and texture of the mushrooms in the field book. Collections were conducted in the morning to ensure the mushrooms' freshness, which was crucial for accurate identification. Visual inspection and reference field guides were employed to identify the specimens, with only edible or medicinal species being selected for further study.

After recording morphological and anatomical details, healthy mushrooms were earmarked for tissue culturing to propagate them in controlled conditions. For the remaining mushrooms, they were dried in a hot air oven at 45 degrees Celsius for 4-6 hours to preserve them for future reference or potential study. This systematic approach ensured a comprehensive and well-documented collection process, laying the groundwork for subsequent studies and tissue culture experiments.

Identification of wild mushroom

Morphological features play a crucial role in mushroom identification. The following

characters were meticulously observed during the study:

Pileus Characters

The pileus, or cap, characteristics such as size, shape, color, and surface texture were documented. Size was determined by noting the diameter and height of both the smallest and largest fruit bodies. Shapes observed included flat, convex, and conical, among others. Colour variations ranging from white to brown or mixed shades were documented. Surface characters of the pileus were observed for texture, margin, cuticle, and context. The flesh's taste and odour were described, ranging from mild to acrid or fragrant to disagreeable. The gill attachment types (adnate, decurrent), shape, width, spacing, and depth (crowded or distant) were noted. The presence or absence of short lamellae (lamellulae) and gill colour, including changes upon bruising, were documented. Regarding the stipe or stem, characteristics such as colour and colour changes upon bruising, shape, size, position, and surface texture (viscid, dry) were observed. Additionally, the presence or absence of veils, like annulus or volva, was noted.

Spore Print Colour

The spore print, a critical feature for taxonomy, was obtained by placing the cap on paper. The resulting spore deposit was then observed for its color.

Tissue culturing steps

Tissue culturing is a technique used to isolate and grow fungal mycelium, the vegetative part of mushrooms, from a small piece of tissue. This allows for the creation of a pure culture, free from contamination by other microorganisms.It involves transferring a small piece of healthy mushroom tissue onto Potato Dextrose Agar (PDA) media in sterile Petri dishes or culture tubes.The plate is then sealed and incubated at the appropriate temperature for the

mushroom species. After a few days, the fungal mycelium will begin to grow out from the tissue explant. The mycelium will continue to grow until it fills the plate.Once the plate is fully colonized with mycelium, it can be subcultured onto fresh agar plates. This helps to maintain the culture and to reduce the risk of contamination. Potato Dextrose Agar (PDA) media, which provides the necessary nutrients for mushroom mycelium to grow.

Potato Dextrose Agar Preparation (PDA)

Materials Needed:

- Potatoes (approximately 200g)
- Dextrose (20g)
- Agar powder (15g)
- Distilled water (1 liter)
- pH meter or pH indicator strips
- Autoclave or pressure cooker
- Stirring rod or magnetic stirrer
- Beakers or flasks
- Sterile containers or petri dishes

Procedure:

For the preparation of potatoes, they were peeled, washed to remove any dirt or debris, and then cut into small pieces or slices. To create the potato extract, these pieces were boiled in distilled or deionized water, ensuring the potatoes were fully submerged. After simmering for approximately 30 minutes to extract the starch and nutrients, the extract was strained through cheesecloth or filter paper to remove any solid potato residues, yielding a clear liquid. This potato extract was fortified by

adding 20g of dextrose and 15g of agar powder. The mixture was stirred thoroughly to ensure complete dissolution of the dextrose and agar. To confirm the suitability of the medium for microbial growth, pH indicator strips were used to measure the pH, aiming for an optimal value of around 5.6 for PDA media.

The prepared PDA media was poured into sterilizable containers or petri dishes, leaving some space at the top to allow for agar expansion. The lids were closed loosely to allow steam to escape during sterilization. The containers were placed in an autoclave or pressure cooker and sterilized at 121°C (250°F) for 15-20 minutes at 15 psi (1.05 kg/cm^2). The media was allowed to cool down to around 50-55°C before being poured into plates or tubes. Once the media cooled down to the desired temperature, it was poured into sterile petri dishes or test tubes.

Preliminary Phytochemical analysis

One gram of dried and finely powdered plant material was placed in a conical flask and mixed with 100 ml of water. The flask was then placed on a shaker for 24 hours. After shaking, the water extract was filtered through filter paper to remove any solid particles or residues. The water extract was stored in a refrigerator when not in use. The resulting filtrate was used for phytochemical analysis according to standard methods (Shaikh and Patil, 2020)

Test for proteins

Millon's Test: Upon mixing the crude extract with 2 ml of Millon's reagent, a white precipitate formed. Gentle heating of the mixture resulted in the precipitate turning red, confirming the presence of proteins.

Test for carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to the crude extract and gently boiled . A brick red precipitate appeared at the bottom of the test tube indicating the presence of reducing sugars.

Benedict's test: When the crude extract was mixed with 2 ml of Benedict's reagent and boiled, a reddish-brown precipitate formed, indicating the presence of carbohydrates.

Test for Phenols and Tannins: Crude extract was mixed with 2ml of 2% solution of FeCl2 .A blue -green or black colouration indicated the presence of phenols and tannins.

Test for Flavonoids

Alkaline reagent test: Crude extract was mixed with 2ml of 2% solution of NaOH.An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids

Test for cardiac glycosides

***Keller -kilani test*:** Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2 % solution of FeCl3.The mixture was then poured into another test tube containing 2ml of concentrated H2SO4 .A brown ring at the interphase Indicated the presence of cardiac glycosides

Test for Saponin

Foam test: Shake the extract with water. Presence of saponins produces foam. Persistent foam indicates a positive result, suggesting the presence of saponins in the sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Collection of Wild Mushrooms

NIne mushrooms were collected and identified.. The Photos sare in Figure 1 and details of their family and ecology, place of collection etc are listed in Table 1

Trametes versicolor

Many zoned polypore with various colors (cream and brown). Caps 2.5-8 cm wide and 1-3 mm thick. Multicolored with yellowish and orangish brown concentric zones; outermost zones usually pale. white to yellowish pores on the underside of its fan-shaped, wavy-edged cap. Spore print white. Edibility**:** Inedible.

Auricularia auricula

Auricularia auricula, also known as wood ear, is a fungus with distinctive brown, gelatinous basidiocarps that resemble an ear in shape. These fungi grow on the wood of older trees. The fruit body can reach sizes of up to 90 mm across and 3 mm thick. The upper surface of fruit bodies has tiny hairs and a folded, wrinkled appearance. Edibility**:** Edible.

Auricularia delicata

The fruit bodies of *Auricularia auricula* are ear-shaped, reaching sizes of up to 120 mm across and 5 mm thick, in clusters. The spore-bearing underside is smooth and wrinkled.

While they commonly grow on dead wood, they can also be weakly parasitic on living trees. It is edible.

Calocera cornea

Tiny fruit bodies usually shorter than the thickness of a finger can be seen on trunks and twigs.The fruit bodies are gelatinous and rubbery yellow in color. The spore print is white or very pale yellow Edibility**:** Inedible.

Coprinellus disseminatus

Commonly known as the fairy inkcap, this fungus forms dense masses that swarm over rotting tree stumps and roots. The cap typically ranges from 0.5 to 1.5 cm in diameter and starts off around 1-1.5 cm tall. Initially, it appears in an egg shape, but as it matures, it turns into a bell shape. The spore print of this fungus is black in color. Edibility**:** Inedible.

Ganoderma lucidum

Ganoderma lucidum is a large, dark mushroom with a glossy exterior and a woody texture. One distinguishing feature of Ganoderma from other Polypores is its double-walled basidiospores. The fruit bodies of Ganoderma grow in a fan-like or hoof-like form on the trunks of both living and dead trees.

Pleurotus djamor

Pleurotus djamor, commonly known as the pink oyster mushroom, features fan-shaped caps that are typically 3-7 cm long with an inrolled margin. The gills of this mushroom range from light pink to cream in color. Its texture is both meaty and chewy, making it a unique culinary ingredient. Edibility: It is edible.

Pleurotus flabellatus

It is an oyster mushroom with fan-shaped caps, range from white to light brown with creamy white to pale yellow gills. It has a firm, tender texture and mild flavor. The gills are creamy

white to pale yellow and run down the short stalk.The spore print is typically white to pale yellow. Edibility: It is edible.

Trametes betulina*Trametes betulina* are known as gilled Polypores. The fruiting body have gills instead of pores, which distinguishes *Trametes versicolor.* It is multicolored, cap is about 1-10 cm and the stem is absent. It's seen in leather texture. Spore print is white. Inedible

Table 1. Details of Collected Mushrooms

Mushroom	**Ecology**	**Colour**	**Texture**	**Habit**	**Gill/Pore**	**Place of Collection**
Auricularia auricula	Live trees, decaying wood	Dark brown	Gelly	Clustered	No gills/pore	Thattekkad
Auricularia delicata	On decaying wood	Light brown to pinkish	Gelly-rubbery	Clustered	No gills/pore	Thattekkad
Calocera cornea	On decaying wood	Bright yellow	Gelly	Group	No gills/pore	Thattekkad
Coprinellus disseminatus	Damp areas with decaying wood	Gray	Thin and fragile	Group	Gill	Ernakulam
Ganoderma lucidum	Dead or dying trees	Reddish-brown and yellow ring	Woody	Solitary	Pore	Thattekkad
Pleurotus djamor	On dead wood	Pink	Soft	Clustered	Gill	Idukki
Pleurotus flabellatus	On dead wood	White	Soft	Clustered	Gill	Thattekkad
Trametes betulina	On dead and decaying	White	Leathery, tough	Solitary	Gill	Ernakulam

	wood					
Trametes versicolor	On dead wood	Multi coloured rings	Leathery	fused rosettes	Pore	Thattekkad

Figure 1. Collected Mushrooms

Medicinal Properties of the Collected Mushrooms

Mushrooms have diverse medicinal properties, from immune-boosting to anti-inflammatory effects. Bioactive compounds within, like polysaccharides and antioxidants, drive these benefits. Scientific research validates many traditional uses, making mushrooms promising in both traditional and modern healthcare. The following table summarises the medicinal properties of collected wild mushrooms from literature (Pan et al., 2019;Cai et al., 2015; Islam et al., 2021; Deepalakshmi and & Mirunalini 2011; Maity et al.,2021; Deepalakshmi and Sankaran, 2014; Frljak et al., 2021).

Mushroom Species	Medicinal Properties
Auricularia auricula-judae	Cardiovascular health, immune system support, digestion aid, anti-inflammatory, antioxidant
Auricularia delicata	Limited research, Potential antioxidant and immune-boosting properties
Calocera	Antimicrobial, wound-healing (Traditional uses)
Coprinellus disseminatus	Antioxidant, antimicrobial (Limited research)
Ganoderma lucidum	Immune-modulating, anti-inflammatory, liver health support, diabetes management
Pleurotus djamor	Immune-boosting, cardiovascular health support
Pleurotus flabellatus	Antioxidant, anti-inflammatory, antimicrobial, diabetes management, liver protection
Trametes betulina	Immune-boosting, anticancer, antioxidant
Trametes versicolor	Immune-modulating, anticancer, contains PSP and PSK for immune function support

4.3 Tissue culture Preparation of Wild Mushrooms

Out of nine mushroom species collected from the wild and attempted for culturing, only three were succeeded: *Trametes betulina, Pleurotus djamor,* and *Auricularia delicata.* The culturing was done in Potato Dextrose Medium (PDA). Mycelial growth was observed using

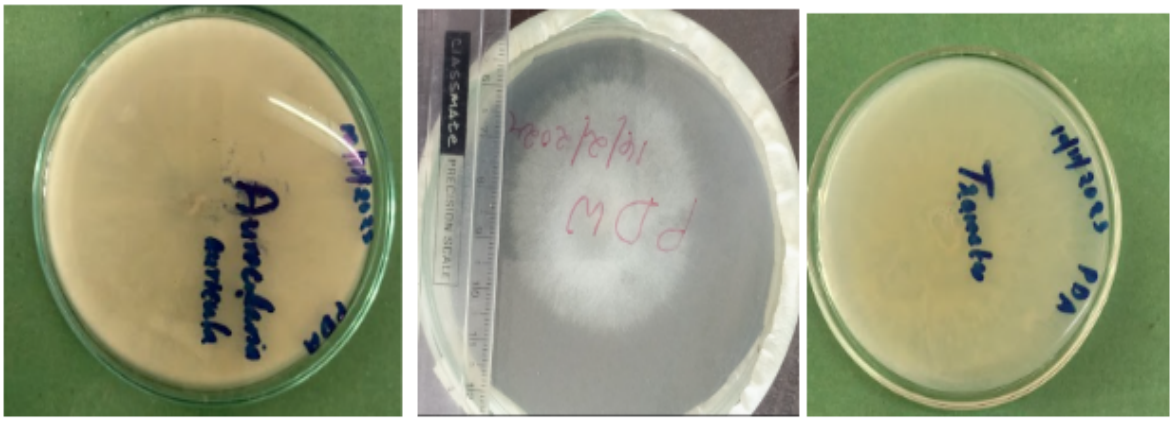
a ruler across the Petri-dish horizontally. The growth rate calculation is given by the formula below: Growth rate = Colony diameter on the last day (cm) /Number of day's measurement was taken after inoculation.

Details of their growth and mycelium are shown in table 2.

Table 2 Mycelial characteristics in PDA medium

Mushroom	**Mycelium characteristics**			**Temperature in degrees**
	Colour	**Texture**	**Growth**	
Auricularia delicata	White with pinkish tint	Wooly	Fast	20-30
Pleurotus djamor	White	Cottony	Slow	20-30
Trametes betulina	Off White	Cottony	Moderate	20-30

Mycelial observations revealed distinct characteristics among the three fungi. *Auricularia delicata* stood out with its unique white coloration tinged with pink and a wooly texture, suggesting a fluffy appearance. This species displayed the fastest growth rate, hinting at its adaptability and vigor under the culturing conditions. In contrast, *Pleurotus djamor* exhibited a white, cottony mycelium, but its growth rate was slower compared to the others. This might reflect its natural growth pattern or specific nutrient requirements.*Trametes betulina* showed moderate growth, balancing between the other two. The pure cultures of mushrooms are shown in figure 2.



Auricularia delicata *Pleurotus djamor* *Trametes betulina*

Figure 2 Pure cultures of *Au,ricularia delicata, Pleurotus djamor* and *Trametes betulina*

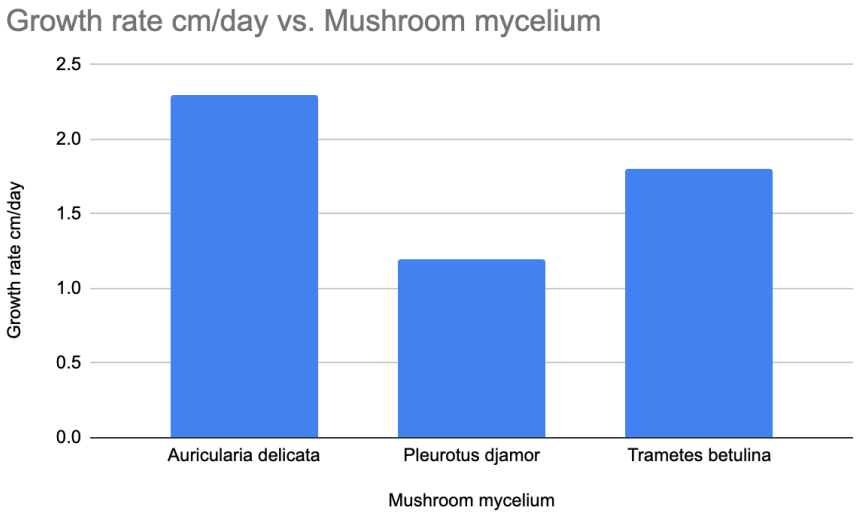


Figure 3. Comparative Growth rate of mushroom mycelium in PDA Medium

The cultivation of basidiomycetes, including wild mushrooms, requires careful attention to various physicochemical conditions to optimize mycelial growth and biomass production. Krupodorova et al. (2021) emphasized the variability in cultivation conditions required by different basidiomycete species and even different strains of the same species. Our findings on *Trametes betulina, Pleurotus djamor, and Auricularia delicata* align with this variability, highlighting the need for species-specific cultivation protocols.

Temperature plays a crucial role in fungal growth. Our results showed that the optimal temperature for *Auricularia delicata'*s mycelial growth was between 25~30°C. Similarly, the study by Krupodorova et al. (2021) highlighted the significance of temperature in influencing mushroom growth, suggesting that different species might have distinct temperature preferences.

Auricularia delicata demonstrated the fastest growth rate of 2.3 cm/day, followed by *Trametes betulina* at 1.8 cm/day and *Pleurotus djamor* at 1.2 cm/day. These differences in growth rates might be attributed to their inherent biological characteristics, including mycelial structure, metabolic rates, and nutrient requirements (Abdel Aziz et al., 2018)

4.4 Phytochemical Analysis of *Pleurotus djamor*

The phytochemical analysis of Pleurotus djamor showed positive results for proteins (++), strong positives for carbohydrates (+++), and presence of phenols (+), tannins (+), glycosides (+), alkaloids (+), and flavonoids (+). However, saponins were absent (-).

The phytochemical analysis of *Pleurotus djamor* and related species reveals a diverse composition of bioactive compounds, including phenols, flavonoids, saponins, tannins, and terpenoids . These compounds are known for their various pharmacological activities, such as

antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects. Phenolic compounds and flavonoids, in particular, are highlighted in both studies for their antioxidant properties, contributing to the overall free radical scavenging activity of *Pleurotus djamor* (Sasidhara and Thirunalasundari , 2014.; Acharya et al. 2017).

Table 3 Priliminary Phytochemical screening of *P.djamor*

Test	**Result**
Protein -Millon's test	++
Carbohydrate- Benedict	+++
Test for Phenols	+
Tannins	+
Glycoside Keller -kilani test	+
Saponin	-
Alkaloid -flavonoids	+

CHAPTER 5

SUMMARY AND CONCLUSION

The study explored nine wild mushroom species, characterizing them based on morphology, ecology, and medicinal properties. While some, like *Auricularia auricula* and *Pleurotus species,* are edible and have potential health benefits ranging from immune-boosting to anti-inflammatory effects, others like *Trametes versicolor* and *Calocera cornea* are inedible or toxic. The research showed different mushroom species require specific conditions for growth, and analyzed the promising medicinal properties of one cultivated species. Tissue culture experiments revealed distinct mycelial growth characteristics among *Trametes betulina, Pleurotus djamor*, and *Auricularia delicata*, with *Auricularia delicata* showing the fastest growth. Phytochemical analysis of *Pleurotus djamor* identified bioactive compounds such as proteins, carbohydrates, and phenols, highlighting its potential pharmacological activities.

Future research could involve optimizing cultivation methods for all the mushrooms. Further investigation into the bioactive compounds identified through chemical analysis is required. The study recommends exploration of the remaining wild mushroom species for their potential health benefits and properties

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