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Cultivation of Insights of Bioactive Compounds Analysis of *Pleurotus florida* (Fr.) Kumm and *Hypsizygus ulmarius* (Bull.:Fr.)

Abirami Govintharajan*[1], Gomathi Selvam[2], Ambikapathy Varatharaju[3] and Panneerselvam Annamalai[4]

[1-4] P. G. and Research Department of Botany, A. V. V. M. Sri Pushpam College (Autonomous) (Affiliated to Bharathidasan University, Trichy), Poondi - 613 503, Thanjavur (District), Tamil Nadu, India

Abstract

Mushroom cultivation is one of the most profitable business and environment friendly enterprises, with the various horticultural crops in India. In the current investigation focused on the cultivation of edible mushroom with two different agricultural waste substrates were performed. The two types of raw materials such as paddy straw and sugar cane trash were used for cultivation of *Pleurotus florida* and *Hypsizygus ulmarius.* Growth and yield parameters of *P. florida* was initiated from I, II and III harvested stages were observed. The first harvest mushroom in both was excellent growth and weight when compared with the other two harvests. The total yield of edible mushrooms in paddy straw was maximum production than that of sugarcane trash respectively whereas *H. ulmarius* mushroom was moderate growth yield were performed. The biological efficiency of *H. ulmarius* grow with paddy straw was maximum yield found to be recorded than sugarcane trash substrate respectively. However, the paddy straw substrate was excellent for cultivation of mushroom when compared with sugarcane trash substrates. Maximum fruit bodies were harvested when *P. florida* were cultivated in paddy straw substrates Qualitative insights of bioactive compounds such as alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, phlobatannins, quinones, saponin, steroids, tannin and terpenoids were screened from *P. florida* and *H. ulmarius* with four different solvents were used for extraction. Among the four solvents extracts of the diethyl ether using *P. florida* showed maximum bioactive compounds like alkaloids, amino acids, coumarines, glycoside, phenols, phlobatannins, quinones, saponin, steroids, tannin and terpenoids recorded respectively. These bioactive compounds are responsible for many biological properties in our day today life. Hence, these bioactive compounds are responsible for many nutraceutical properties are described in mushrooms for prevention of various diseases including hypertension for the human era.

***Key words*:** *Pleurotus florida, Hypsizygus ulmarius,* Edible mushroom, Bioactive compounds, Solvents, Substrates

Research on the therapeutic properties of oyster mushrooms started in the late 20th century. First, their hypertensive properties were confirmed [1]. *Pleurotus* mushrooms are regarded as being nutritious due to their abundance in proteins, fibre, vitamins, and minerals because of their tasty flavour, pleasant perfume, and medical benefits, *pleurotus* mushrooms are used as a functional food [2]. Oyster mushrooms, or *Pleurotus* species, are widely cultivated and have significant commercially important and [3]. Oyster- or shell-shaped, and in a variety of hues including white, cream, grey, yellow, pink, and light brown are the basidiocarps of oyster mushrooms [4]. They have been chiefly used in traditional medicine in China and other Asian countries [5]. It has economic and ecological values and medicinal properties. The substrate used for their cultivation does not require sterilization, only pasteurization, which is less expensive. Growing oyster mushrooms convert a high percentage of the substrate to fruiting bodies, increasing profitability. *Pleurotus ostreatus* demands few environmental controls and their fruiting bodies are not often attacked by diseases and pests and they can be cultivated in a simple and cheap way. All this makes *Pleurotus ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms. The majority of lignocellulosic substrates and other wastes produced by the agricultural, forestry and food-processing industries are capable of being colonized and degraded by edible mushrooms. Particularly when compared to other edible mushrooms, *Pleurotus ostreatus* requires a shorter growing period. The substrate used for their cultivation just needs to be pasteurized, which is less expensive, rather than sterilized [6].

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Correspondence to: Abirami Govintharajan, P. G. and Research Department of Botany, A. V. V. M. Sri Pushpam College (Autonomous) (Affiliated to Bharathidasan University, Trichy), Poondi - 613 503, Thanjavur (District), Tamil Nadu, India; Tel: +91 9344170854; E-mail: sanmugapriya2320@gmail.com

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The substrates that have been used for mushroom production in previous studies include rice straw, rice bran, wheat straw, pulp, corncobs, cocoa shell waste, cotton waste, spent grain, sawdust, maize husks, and cassava peelings [7-8]. Other substrates are soybean straw, paddy straw, sun flower stalks, sugarcane bagasse, fruit waste, used tea leaves, bamboo leaves, and maize stalk [9-11]. More recently, [10] elaborated on the bioactivities of polysaccharides from *Pleurotus* species and the development of new extraction methods. The bioactive compounds identified in *Pleurotus* mushrooms can be divided into those with a high molecular weight and those with a low molecular weight [11].

MATERIALS AND METHODS

Sample collection site

In the present study *Pleurotus florida* and *Hypsizygus ulmarius* strain isolated samples obtained from fresh, healthy spawn MM spawn lab GSP mushroom farm, Karanthai, Thanjavur, Tamil Nadu, India. The mycelia form of colonies of different strains was maintained on PDA medium where inoculated in to 100 ml potato dextrose broth contained in 250 ml conical flask. After four weeks growth to the fungus in the liquid medium were observed. Another type of cultures was maintained from Petri plates and test-tubes are used in the culture was maintained potato dextrose agar (PDA) medium.

Mushroom cultivation [12]

Culture media

The mushroom strains can be maintained in semi synthetic solid medium. Potato Dextrose Agar (PDA) is the most commonly used media for maintaining the inoculums and they showed good growth rate.

Potato dextrose agar (PDA) medium composition

Dextrose 20.00g peeled potato pieces 250.00g agar 18.00g distilled water 1000 ml pH 7.0 to 7.5.

About 250gms of Potato tubers were washed, peeled off, cut into small piece and taken in a 500 ml conical flask containing 300ml of water. It was boiled for about half an hour and the extract was decanted. About 18gm of agar shreds were weighted, taken in another conical flask and 500ml of distilled water was added. The agar was melted over a heater. The molten agar was then mixed with the potato extract and made up to 1 litter. Then 15gm of dextrose was added to the medium and mixed thoroughly. Since the growth of the fungus is flavoured by acidic range the pH was adjusted to 5-6. The medium was distributed in 250ml conical flask plugged with cotton and sterilized in an autoclave. To avoid bacterial growth by streptomycin sulphate was added.

Culturing of Pleurotus florida and Hypsizygus ulmarius mycelium on petri plates

Inoculation technique

The sterilized bottles are placed in the culture room. The UV lamp is switched on for 15 minutes to sterilize the air inside and then surface was cleaned using alcohol to ensure axenity before the use. The growing edge of the fungi from Petri plate was cut with the help of a cork borer and transferred to the spawn bottle in front of the flame. The bottles are incubated at room temperature. The white mycelium is observed in the entire bottle after 12 days of inoculation. This is known as 'mother spawn'.

Substrates

Mushroom beds can be prepared using Paddy straw and sugarcane trash substrates. One spawn bottle can be used to prepare two beds. Size of the bed should be about 30 × 60 cm.

Bed preparation

Fresh substrates are chopped into pieces of 2-3 inches length and soaked in water for 10 hours. Water is then drained off from the substrates. Afterwards, the substrates are sterilized using vertical autoclave at 15 lbs pressure for 20 minutes. The sterilized substrates are placed on a wire mesh net for draining excess water. Polythene covers in the size of 30 × 60cm are procured and filled with the treated substrates. Before preparing mushroom beds hands and all the instruments should be sterilized with a dilute solution of $KMnO_4$/ alcohol. A polythene bag is tied at one end and sterilized substrates are filled through the open end for about 5cm in length. A handful of spawn from the bottle is spread (15g) towards the periphery of this layer. Over the spawn some more substrates are put and pressed lightly. This process is repeated five times. The mouth of the bag is rolled and closed with stapler pins. Holes are made over the bag for aeration. Inoculated paddy straw bags are kept in a ventilation dark chamber. The mycelia will colonize the entire paddy straw bag within 15 days. Now the polythene cover is peeled off and the compact lump of paddy straw is placed in a cool shady room and sprayed with water 3-4 times per day. The young fruit bodies will come out from the bag. When the fruit bodies attain full growth, they could be harvested.

Bioactive compounds screening of mushrooms

Phytochemical analysis was carried out for solvents of mushrooms as per standard methods [13] but with some little modifications.

Preparation of extracts [14-16].

After collection, the mushroom samples were wrapped in newspaper and stored in moisture-free open spaces. The removal of all foreign matters was done. Then, they were ground using a metal mortar and pestle. The powder was collected and ground again at the end. The bioactive components of oyster mushrooms were determined using standard method.

Detection of bioactive components

The bioactive component analysis was done using standard method [17].

Qualitative bioactive compounds analysis [18].

Freshly prepared different solvents extracts were tested for bioactive compounds using standard methods. The bioactive compounds such as alkaloids, amino acids, coumarins, flavonoids, glycoside, phenols, phlobatannins, quinones, saponins, steroids, tannins and terpenoids were analyzed with the solvents of aqueous, ethanol, methanol and diethyl ether extracts.

Quantitative bioactive analysis

Preliminary bioactive substances like alkaloids 18, aminoacids 18, coumarins 18, flavonoids 19, glycoside, phenols, phlobatannins, quinones 18, saponins 20, steroids 18, tannins 21 and terpenoids18 were analyzed by using standard methods.

Statistical analysis

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

RESULTS AND DISCUSSION

Grown on a variety of crop residues as substrates were oyster mushrooms, *P. florida* and *H. ulmarius.* (Table 1-2).

Spawn running phase

The ideal primordial initiation days varied between species and substrates, ranging from 15-20 days for the full spawn run of *P. florida* on paddy straw to 17-25 days on sugarcane trash for *H. ulmarius*. The least optimal growth period was recorded at 16 and 24 days for *P. florida* and *H. ulmarius*, respectively, when using paddy straw as the substrate. This difference was found to be statistically significant, indicating that the specific substrate used had an effect on the primordial initiation period. Additionally, the difference between the two species was also found to be statistically significant, suggesting that there are differences between them in terms of their optimal growth periods.

Table 1 Effect of different agricultural waste substrates and biological efficiency of *Pleurotus florida*

Substrates	Size of the bag	Spawn run (days)	Pin head formation (days)	Yield per harvest (g/kg)			Total yield (g/kg)
				I	II	III	
Paddy straw	60 × 30 cm	16	19	420	360	260	1,040
Sugarcane trash		18	21	380	260	220	860

Table 2 Effect of different agricultural waste substrates and biological efficiency of *Hypsizygus ulmarius*

Substrates	Size of the bag	Spawn run (days)	Pin head formation (days)	Yield per harvest (g/kg)			Total yield (g/kg)
				I	II	III	
Paddy straw	60 × 30 cm	22	25	360	273	220	853
Sugarcane trash		24	28	340	240	190	770

Pin head formation

Among sugarcane trash combinations, the maximum number of days between pinhead formation and fruiting body formation varied significantly (21 and 28 days for *Pleurotus florida* and *Hypsizygus ulmarius*, respectively). On paddy straw, the shortest growth periods for the development of fruiting bodies were observed (19 - 25 days for *Pleurotus florida* and *Hypsizygus ulmarius* respectively).

Table 3 Qualitative analysis of bioactive compounds of *Pleurotus florida*

Bioactive compounds	Different solvents			
	Aqueous	Diethyl ether	Ethanol	Methanol
Alkaloids	+	+	+	+
Amino acids	+	+	+	+
Coumarins	+	+	-	-
Flavonoids	+	-	+	+
Glycosids	+	+	-	+
Phenols	+	+	+	+
Phlobatannins	+	+	+	+
Quinones	+	+	-	-
Saponin	-	+	-	-
Steroids	+	+	+	+
Tannin	+	+	-	-
Terpenoids	-	+	-	+

(+) Present (-) Absent

Table 4 Quantitative analysis of bioactive compounds of *Pleurotus florida*

Bioactive compounds	Quantity (mg/g)			
	Aqueous	Diethyl ether	Ethanol	Methanol
Alkaloids	1.05 ± 0.03	1.02 ± 0.06	1.10 ± 0.03	1.09 ± 0.06
Amino acids	2.02 ± 0.23	2.31 ± 0.00	1.63 ± 0.36	1.36 ± 0.30
Coumarins	1.03 ± 0.00	1.03 ± 0.23	-	-
Flavonoids	1.10 ± 0.07	-	1.69 ± 0.00	1.49 ± 0.09
Glycosids	1.03 ± 0.23	1.26 ± 0.20	-	1.00 ± 0.00
Phenols	1.51 ± 0.01	1.26 ± 0.08	1.38±0.03	1.44 ± 0.43
Phlobatannins	1.66 ±0 .22	1.30 ± 0.02	1.00±0.00	1.02 ± 0.36
Quinones	1.00 ± 0.02	1.25 ± 0.63	-	-
Saponin	-	1.16 ± 0.03	-	-
Steroids	1.45 ± 0.00	1.75 ± 0.05	1.74±0.03	1.17 ± 0.09
Tannin	1.36 ± 0.00	1.65 ± 0.63	-	-
Terpenoids	-	1.63 ± 0.02	-	1.36 ± 0.36

Standard deviation ± error

Fresh weight of fruiting bodies

On paddy straw, *Pleurotus florida* and *Hypsizygus ulmarius* harvested mushrooms had a maximum fresh weight of 420g and 360g, respectively, and a minimum fresh weight of 380g and 340g, respectively, from the sugarcane waste. On paddy straw, harvested mushrooms had a maximum fresh weight of 360g and a minimum fresh weight of 240g for *Pleurotus florida* and *Hypsizygus ulmarius*, respectively. The highest fresh weight of mushrooms from the third harvest (260g and 220g of *Pleurotus florida* and *Hypsizygus ulmarius*) was

recorded on paddy straw and the lowest (220g and 190 g of *Pleurotus florida* and *Hypsizygus ulmarius*) was recorded from the sugarcane trash respectively.

Paddy straw recorded the maximum fresh weight of harvested mushrooms (*P. florida* and *H. ulmarius*) (420 g and 360 g), whereas sugarcane trash recorded the minimum fresh weight (380 g and 340 g) (Table 3-4).

Bioactive compounds of P. florida and H. ulmarius

The present study was carried out using a preliminary bioactive compound analysis of *Pleurotus florida* and *H Hypsizygus ulmarius* with four solvents such as aqueous, diethyl ether, ethanol and methanol were performed.

Diethyl ether and aqueous extracts of *Pleurotus florida* contain bioactive compounds with medicinal uses. Both aqueous and diethyl ether extracts of *Pleurotus florida* contained alkaloids, amino acids, coumarins, glycosides, phenols, phlobatannins, quinones, and steroids. Flavonoids are not present in diethyl ether but they are present in aqueous solutions. Diethyl ether contains saponin and terpenoids that are absent from the aqueous extract. *Pleurotus florida* ethanol and methanol extracts were found to be devoid of some bioactive compounds, such as alkaloids, amino acids, flavonoids, phenols, phlobatannins and steroids as well as coumarins, saponins, quinones and tannins which are usually absent.

Diethyl ether and aqueous extracts of *Hypsizygus ulmarius* revealed the presence of medicinally essential bioactive components. The aqueous and diethyl ether extracts of *Hypsizygus ulmarius* have been located to comprise alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, quinones, steroids and tannin. These are commonly present and saponin is generally absent. Phlobatannins are present in aqueous solutions but are absent in diethyl ether. Terpenoids are found in diethyl ether but are absent from the aqueous extract. *Hypsizygus ulmarius* ethanol and methanol extracts have been found to contain a few bioactive compounds that are commonly found with alkaloids, amino acids, flavonoids, phenols, phlobatannins, and steroids, but coumarins, glycosides, quinones, and saponins are usually absent. The ethanol extract of *Hypsizygus ulmarius*, which turned into found to be without bioactive compounds, including terpenoids, the methanol extract carries them (Table 5-6).

Table 5 Qualitative analysis of bioactive compounds of *Hypsizygus ulmarius*

Bioactive compounds	Different solvents			
	Aqueous	Diethyl ether	Ethanol	Methanol
Alkaloids	+	+	+	+
Amino acids	+	+	+	+
Coumarins	+	+	-	-
Flavonoids	+	-	+	+
Glycosids	-	+	-	-
Phenols	+	+	+	+
Phlobatannins	+	-	+	-
Quinones	+	+	-	-
Saponin	-	-	-	-
Steroids	+	+	+	+
Tannin	+	+	-	-
Terpenoids	-	+	-	+

(+) Present (-) Absent

Table 6 Quantitative analysis of bioactive compounds of *Hypsizygus ulmarius*

Bioactive compounds	Quantity (mg/g)			
	Aqueous	Diethyl ether	Ethanol	Methanol
Alkaloids	1.05 ± 0.13	1.00 ± 0.15	1.13 ± 0.13	1.19 ± 0.16
Amino acids	4.00 ± 0.03	3.69 ± 0.55	1.13 ± 0.16	2.16 ± 0.10
Coumarins	1.01 ± 3.00	1.36 ± 0.15	-	-
Flavonoids	1.00 ± 0.09	1.36 ± 0.69	2.19 ± 0.10	2.09 ± 0.19
Glycosids	1.03± 0.33	1.23 ± 0.32	-	-
Phenols	1.01 ± 0.11	1.60 ± 0.58	1.08 ± 0.13	1.04 ± 0.43
Phlobatannins	1.06 ± 0.02	-	1.10 ± 0.00	2.12 ± 0.26
Quinones	1.10 ± 0.12	1.87 ± 0.00	-	-
Saponin	-	-	-	-
Steroids	1.05 ± 1.00	1.63 ± 0.36	1.04 ± 0.13	1.10 ± 0.00
Tannin	1.06 ± 3.00	1.47 ± 0.26	-	-
Terpenoids	-	1.69 ± 0.26	-	1.06 ± 0.00

Standard deviation ± error

The oyster mushroom (*Pleurotus ostreatus*) was successfully grown on substrates used for cultivation. [20] reported that agricultural wastes for substrate production and nearly all types of agricultural waste can be used to grow mushrooms. Oyster mushrooms (*Pleurotus ostreatus*) could be grown on corncob, finger millet straw, bamboo waste, and their combinations with varying growth performance. The lowest biological efficiency and fresh weight were recorded from a mixture of corncob and bamboo waste substrates. Finger millet straw was the best substrate in terms of yield and biological efficiency [21]. The four *Pleurotus* species, *P. citrinopileatus*, *P. eryngii*, *P. ostreatus*, and *P. sapidus* showed different mycelial growth rates and colonisation on the different substrates used. It has been indicated that *P. ostreatus* recorded the shortest colonisation time of the two substrates followed by *P. sapidus*. However, *P. eryngii* had the most extensive mycelial colonization followed by *P. citrinopileatus*. Coffee parchment gave the fastest mycelia colonisation time as compared to the mycelia colonisation on coffee husks. However, this may also be due to variations in the structure,

chemical composition and nutrient content of the coffee parchment substrate. The time it took for pinheads (primordials) to form after the spawn run varied between the four mushroom species. *Pleurotus ostreatus* recorded the earliest pinhead formation (20 days), followed by *Pleurotus sapidus* (22 days) and lastly *Pleurotus citrinopileatus* (23 days) and *Pleurotus eryngii* (26 days). This may be due to the variations in extracellular enzyme production and the prevailing mushroom growing conditions since each *Pleurotus* species requires different environmental conditions of CO_2 concentration, relative humidity, and temperature [22].

Pleurotus mushrooms are a good source of bioactive substances. Despite the fact that the number of studies on the health-promoting effects of *Pleurotus* mushrooms has increased dramatically in recent years [23], *Pleurotus ostreatus* and *Pleurotus florida* revealed the presence of major bioactive components such as flavonoids, polyphenols, saponins, triterpenoids and steroids. This result is similar to that of [24]. The bioactive compound such as alkaloids, glycosides, resins and tannins were absent from the methanol extracts. Bioactive compounds found in edible mushrooms are known to play a vital role in promoting health. The absence of alkaloids and glycosides confirms the report [25]. Preliminary bioactive compounds analysis of *Pleurotus florida*. Were determined the ethanol extract of *Pleurotus florida* revealed the presence of medicinally important bioactive ingredients. The aqueous extract of *Pleurotus florida* was found that the alkaloids, flavonoids, terpenoids, steroids and cardiac glycosides whereas ethanol extract of *Pleurotus florida* showed, the presence of alkaloids, flavonoids, terpenoids, saponins, and steroids. The aqueous extract of *Pleurotus florida* was found to be some phytochemical compounds such as phenols, saponins, tannins, quinines, phlobatannins and anthroquinones. The bioactive characters of *Pleurotus florida* investigated in water and ethanol extract were summarised [26].

CONCLUSION

It can be concluded that the yield of *Pleurotus florida* and *Hypsizygus ulmarius* grown on paddy straw and sugarcane trash as substrates. The results revealed that *P. florida* yielded the maximum biological efficiency on the paddy straw substrates followed by *H. ulmarius* which produced the least biological efficiency on the when compared with sugarcane trash substrates. Paddy straw was the best substrate in terms of yield and biological efficiency. However, the paddy straw substrate was excellent substrate candidature for the growth of mushroom growers. The bioactive component analysis of edible mushrooms *P. florida* and *H. ulmarius* revealed the presence of major bioactive components such as alkaloids, amino acids, coumarins, glycosides, phenols, phlobatannins, quinones and steroids. The aqueous and diethyl ether solvents are the most bioactive compounds present in both mushrooms.

Author contributions

Mrs. Abirami Govintharajan were designed and finalized the manuscript of study, Dr. Panneerselvam Annamalai and Dr. Ambikapathy Varatharaju provided valuable suggestions for this work, Dr. Gomathi Selvam was collected samples and analyzed the work and prepared the draft manuscript. All authors read and approve the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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