Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 11 [3]February 2022 : 130-134

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Evaluation of *Pleurotus florida* - The White Oyster Mushroom

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ABSTRACT

Mushrooms belonging to the fungi group is a well-known vegetarian delicacy and are not only cost effective, but also supplements nutrition. Mushrooms possess varied biopharmaceutical compounds and possess 40-49% of proteins. Mushrooms are a major untapped source of potent pharmaceutical products. The present study is about the cultivation of the white oyster mushroom- Pleurotus florida with nutrient supplement and its qualitative and quantitative phytochemical analysis. Pleurotus florida is grown in presence of the nutrient Macrotyloma uniflorum. Layer spawning method adopted. The fruiting bodies of mushrooms harvested, dried and powdered and extraction performed followed by phytochemical analysis.

Keywords: *Pleurotus florida, Pharmaceutical, Phytochemical, Macrotyloma uniflorum*

Received 19.11.2021 Revised 03.01.2022 Accepted 29.01.2022

INTRODUCTION

Mushrooms are a potent functional food and also a source for drug development and nutraceuticals. Mushrooms are gaining popularity as food due to its nutritional value and high protein content. Mushrooms are a blessing in disguise in the field of medicine, food and unemployment In India. Mushrooms are popular as an important bio source of novel secondary metabolites in the present period. Curative properties of mushrooms are used in practice of alternative systems. The chemically diverse and wide spectrum secondary metabolites of mushrooms are explored in traditional medicine and in molecular biology [1]. Lignin and cellulose containing substrates are widely used widely to cultivate Oyster Mushroom. Oyster mushroom cultivation is gaining popularity due to their ability to utilise various lignocelluloses and its ability to grow at wide range of temperatures [2]. The cultivation of Oyster mushroom may be considered as an alternative method of disposal playing an essential role in managing and recycling of organic wastes [3]. Pleurotus species comprises of a category of ligninolytic mushrooms that are edible and with medicinal properties. This also possess key role in environmental and biotechnological applications. The third most important cultivated edible mushrooms belong to Pleurotus species. Pleurotus species has a distinct flavour and aroma, it is cholesterol free and is rich in proteins, fiber, vitamins, minerals and carbohydrates. Pleurotus species are encouraging as medicinal mushrooms as it exhibits properties like antioxidant [4], anti-inflammatory, anti-viral, antibiotic, anti-bacterial, anti tumour [4], anti-cancerous, hypocholesterolic, hematological and immunomodulatory activities [5]. Mushooms possess high protein which is reported to be double that of vegetables, four folds that of oranges and notably higher to wheat [6]. Nutraceutical mushroom is a dried biomass or an extract from fruiting body or mycelium which is used as a dietary supplement in the form of capsule or tablet, and increase the immune response and thereby increase the resistance to various diseases. It may also help in regression of diseases. Mushrooms has 93-95% of water, along with minerals such as potassium, iron, calcium, phosphorous, copper and carbohydrates, proteins and fat. Mushrooms are also proved to have Vitamin B and Vitamin D [7]. Mushrooms are rich in fat soluble vitamins along with ergosterol and is believed to be the only vegetarian source of Vitamin D [8]. The demand for mushrooms are consistently increasing as it is easy to culture, cost effective and also highly potent both in terms of nutrition and

medicine. Overall mushroom cultivation not only helps in supplementing health and in developing nutraceuticals, but also plays an essential role in reduction of environmental pollution.

MATERIAL AND METHODS

SPAWN COLLECTION

The packets of spawn of *Pleurotus florida* were procured from IRTC, Mundur, Palakkad, Kerala.

SUBSTRATES AND PREPARATION

Hay collected and cleaned well to remove the foreign particles like stones, mud etc. It is then soaked overnight in clean water. Quantum sufficient water added to develop the substrate moisture to 75%. The soaked hay is removed from water and is pasteurized for three hours to increase the temperature to 98ºC. Pasteurization process applied to *Macrotyloma uniflorum* – the nutrient supplement.

SPAWNING

The pasteurized materials cooled to 26ºC under sterile conditions. The nutrient supplement is mixed with hay and spawn is laid in alternate layers with the hay in a polypropylene bag. The spawn is laid uniformly over the surface also. The bags are tightly tied at its opened side and is weighed and kept in the dark room for a period of 14 days.

CULTIVATION CONDITIONS AND HARVESTING

The inoculated bags incubated in the cultivation room. For ramification of the mycelia 25-30ºC maintained with 85 ± 5% relative humidity. Mushroom growth recorded daily. On the 15th day, the bags were transferred to a sanitized low light less air room for further growth. The bags are opened at its mouth when, mycelium growth is full and pin-heads appear, to facilitate the fruiting bodies development. When fruiting bodies develop and attain optimal size, they are cut and removed just above the substrate level. Four flushes of harvesting done in an interval of 7-8days. Substrate is upturned after the second flush and regular sprinkling of water performed till the completion of 3rd and 4th flushes. Mushroom yield in all flushes recorded.

PROXIMATE ANALYSIS

Moisture, Protein, total carbohydrate, ash and fat determined with AOAC procedures.

PREPARATION OF THE MUSHROOM EXTRACT

Shade dried the freshly harvested mushrooms and finely powdered.

250 ml of 95% methanol, ethanol, ethyl acetate, hexane and aqueous solvents used to extract 25gms of powder by Soxhlet apparatus. By vacuum distillation, the filtered residue was concentrated to a dry mass. The obtained end product used as mushroom extract.

PRELIMINARY PHYTOCHEMICAL CHARACTERISTICS

Preliminary biochemical tests such as carbohydrates, tannins, saponins, proteins, phenols, flavonoids, steroids, terpenoids, alkaloids, glycosides, cardiac glycosides, resins and fixed oil were carried out on the crude aqueous, methanolic, ethanolic, ethyl acetate and hexane extract using standard procedures described by Trease and Evans and Harborne. As per the colour variation classification as no reaction (-), low (++), moderate (++) and high (+++) is made.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

DETERMINATION OF TOTAL PHENOLIC CONTENTS

0.1ml of each extract taken in test tubes and added distilled water to make up the volume to 1ml. 0.5ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) added sequentially in each tube. After swirling the reaction mixture, the test tubes are placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis performed in triplicate and the results expressed as the catechol equivalents.

ESTIMATION OF TOTAL FLAVONOID CONTENT

0.5 ml extract is mixed with 2 ml of distilled water and subsequently added 0.15 ml of 5% sodium nitrate solution. 0.15 ml of a 10% aluminium chloride solution added after 6 min, allowed to stand for 6 min, then 2 ml of 4% sodium hydroxide solution added to the mixture. Distilled water is added immediately to bring the final volume to 5 ml.

The mixture is homogeneously mixed and let stand for another 15 min. Absorbance of the mixture determined at 510 nm. Catechol used as standard compound for quantification of total flavonoid content.

RESULTS

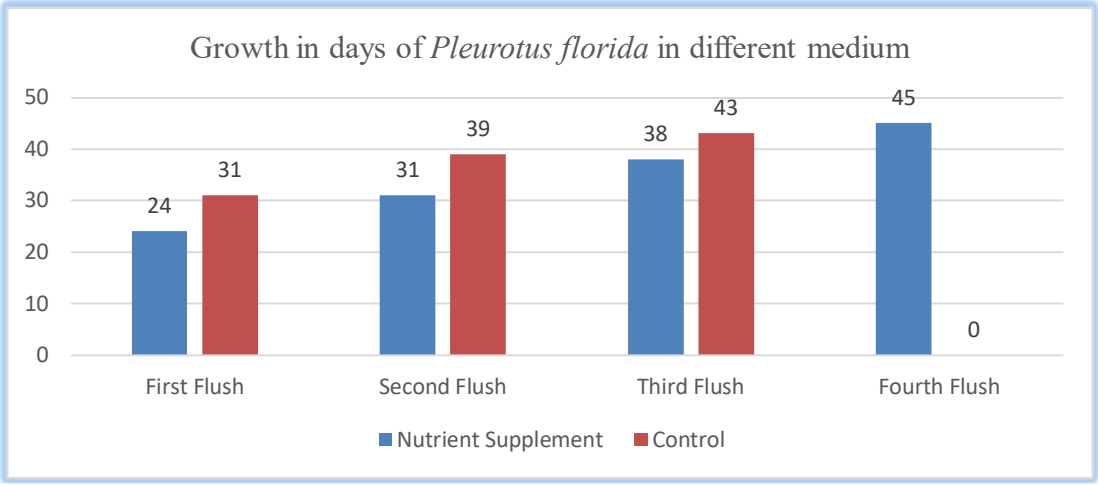
Cultivation of *Pleurotus florida* mushroom on hay substrate performed in two different batches. One batch with *Macrotyloma uniflorum* - nutrient supplement and the second is control batch with substrate alone. The total period of cultivation was for 45days, during which four flushes were obtained. Faster mycelial growth and pin head development observed in the nutrient supplemented batch, i.e., 17th day in the nutrient supplemented batch and on 21st day in the control batch. Time for first flush in control batch was

31days where as that of nutrient supplemented batch was 24days which is much earlier compared to the control batch. The first flush in control batch obtained on 31st day, the second on 39th day, the third on 43rd day and there was no growth for the fourth flush. On the other hand, in the nutrient supplemented batch, the first flush on 24th day, the second on 31st day, the third on 38th day and fourth flush on 45th day (Table1, Fig 1).

Table 1Growth in days of *Pleurotus florida* in different medium.

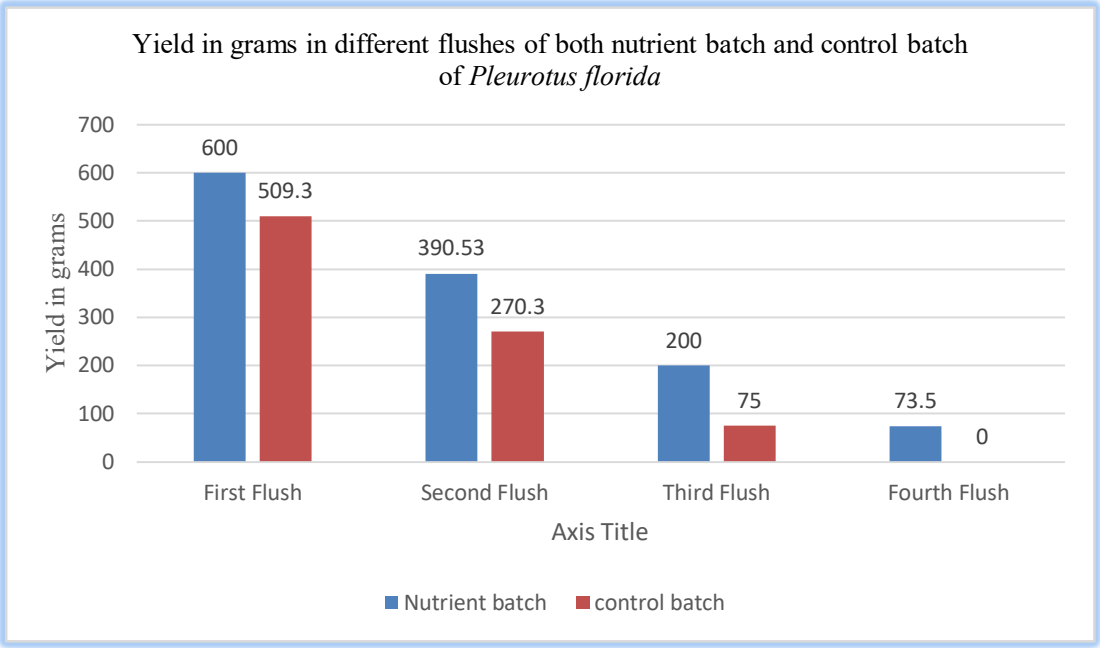
Sl.No	Parameters	Nutrient Supplement Batch (Days)	Control Batch (Days)
1	Primordia Initiation	17	21
2	First Flush	24	31
3	Second Flush	31	39
4	Third Flush	38	43
5	Fourth Flush	45	0

Fig 1: Growth in days of *Pleurotus florida* in different medium



There is a considerable difference in the yield in all flushes noted in both nutrient medium batch and in control batch. The nutrient medium had a yield of 600gms in first flush, 390.53gms in the second flush, 200gms in the third flush and 73.50gms in the fourth flush. The control batch showed the yield of 509.3gms in the first flush, 270.30gms in the second flush, 75gms in the third flush and no yield in the fourth flush (Fig 2).

Fig 2: Yield in grams in different flushes of both nutrient batch and control batch of *Pleurotus florida*



Fresh mushroom contains 88.5% moisture, 55.3% protein, 0.5% fat, 1.3% ash content and 27.1% total carbohydrate concentration. Presence of proteins, carbohydrates, phenolic compounds, glycosides, terpenoids, tannins, steroids, saponins, fixed oils and flavonoids in methanolic and aqueous extracts of *Pleurotus florida* found in the preliminary phytochemical analysis (Table 2).

By the Folinciocalteau method the total phenolic content of *Pleurotus florida* estimated as 61.94mg catechol equivalent in 100ml(10mg/ml). The methanol extract showed higher content of total phenols compared to ethanol and aqueous extracts. The aqueous extract showed 58.1mg whereas the ethanol extract had 59.5mg of total phenol contents. The aqueous extract exhibits 15.31mg of total flavonoid where as ethanol extract showed 15.87mg, hexane extract 7.1mg and ethyl acetate extract contains 8.9mg of total flavonoid content estimated by Aluminium chloride technique.

Table 2 Bioactive compounds found in *P florida* grown in nutrient batch

Type of extract	Protein	Carbo hydrate	Glycoside	Saponin	Steroid	Alkaloid	Tannin	Terpe noids	Fixed oils	Phenols	Flavonoids	Resins
Aqueous	+++	++	+++	+	+	-	+	++	+	+++	++	-
Ethanol	+++	++	++	+	+	+	+	+	++	+++	++	-
Methanol	+++	++	++	+	+	-	+	+	++	+++	++	-
Ethyl acetate	++	+	++	+	+	-	+	+	+	++	-	-
Hexane	++	+	+	+	-	-	-	-	+	++	-	-

DISCUSSION

The primordial initiation in the nutrient supplement batch was on 17th day where as that of control batch was on 21st day in the present study. The growth in nutrient batch was faster than the control batch. The first flush of the nutrient batch was harvested on 24th day which yielded the highest of 600gms followed by the second flush on 31st day yielding 390.53gms, third flush on 38th day yielding 200gms and the fourth flush on 45th day yielding 73.50gms. The control batch exhibited the first flush on 31st day harvesting 509.30gms of oyster mushrooms succeeded by the second flush on 39th day yielding 270.30gms, third flush on 43rd day reaping 75gms and no flush obtained later. An increased yield of 32% noticed in the nutrient supplement batch in comparison to the control batch. The nitrogen deficit substrates are added with protein rich supplements to enhance the quality of the produce. The nutrient supplement batch shows the presence of proteins in aqueous, ethanolic, methanolic, ethyl acetate and hexane extracts. Carbohydrates, glycosides, phenols, fixed oils and saponins were also found in aqueous, ethanolic, methanolic, ethyl acetate and hexane extracts. Steroids, tannins and terpenoids were absent in hexane extract where as their presence were found in aqueous, ethanolic, methanolic, ethyl acetate extracts. Alkaloids were found only in ethanolic extract. Resins were totally absent in all extracts where as presence of flavonoids detected in aqueous, ethanolic and methanolic extracts. Previous studies reveal *Macrotyloma uniflorum* as an excellent source of energy, protein(17.9–25.3%), low content of lipid (0.58–2.06%), carbohydrates (51.9– 60.9%), essential amino acids and a very good source of iron and molybdenum [9]. The moisture content of fresh mushrooms may vary according to the nature of mushrooms, difference in the growth factors of the environment like relative humidity of metabolic water, temperature [10] etc. In the present study, 88.5% moisture content found. Drying by eliminating moisture content of mushroom may increase the nutrient concentration relatively. The drying process of mushrooms extends the shelf life by decreasing biochemical reactions like lipid oxidation and enzymatic browning which results in deterioration of the quality. The type of mushroom, location, nitrogen level, stage of development etc influence the protein content in different edible mushrooms [11]. Flavonoids acts as radical scavengers and is referred as nature's biological response modifiers. Flavonoids has an inherent ability to modify reactions to virus and allergies and exhibits anti-microbial, anti-inflammatory, anti-allergic and anti-cancer activities [12]. Mushrooms being rich in proteins and possess various glycosides, steroids, phenols and flavonoids, is not only an excellent nutraceutical, but also a very powerful pharmaceutical.

CONCLUSION

Cultivation of oyster mushroom in a nutrient supplement like *Macrotyloma uniflorum* not only improves the quality, but also improves the quantity of the yield. *Pleurotus florida* cultivation does not demand high

expenditure as it can be grown in a temperature of 25-30^{0}C and provides employment and is also an alternative method in recycling organic wastes.

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CITATION OF THIS ARTICLE

Smitha Nair M.K., Rekha Parmar, Anurag Talwar, Sabitha Nair M K. Phytochemical Evaluation of *Pleurotus florida* - The White Oyster Mushroom. Bull. Env. Pharmacol. Life Sci., Vol 11[3] Feb 2022 : 130-134.