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Comparison of Yield, Nutrient Content and Antibacterial Activities of Wild and Cultivated Isolates of *Pleurotus djamor*

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ABSTRACT

Background: *Pleurotus* species constitutes one of the choicest edible mushrooms, it is commonly known as "Oyster Mushroom" and in India it is commonly called as "Dhingri. It has important medicinal, biotechnological properties and environmental applications. Its cultivation can be done on a number of agricultural wastes and organic waste materials. There are significant problems in classifying *Pleurotus* isolates using only morphological characters which are often unreliable and inconclusive mainly due to the large influence exerted by environmental factors.

Methods: A wild isolate of *Pleurotus* was collected from North western Himalayas and its identity was confirmed by molecular studies. Yield, nutritional components and its bioactive molecules were compared with the cultivated isolate of *Pleurotus djamore* Antibacterial activities of both the isolates of were performed against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion.

Result: Molecular studies confirmed the identity of wild isolate of *Pleurotus* as *Pleurotus djamore.* The maximum yield of wild isolate was on paddy straw followed by wheat straw. The protein content was 32.3±0.50g^{-1} in wild isolate and 28.7±1.67g^{-1} in cultivated isolate. Presence of nutritional components and phytochemicals inferred in this study indicate the importance of *Pleurotus djamore* in the pharmaceutical.

Key words: Nutritional attributes, Phytochemicals, Pink oyster, *Pleurotus djamore,* Substrates, Yield.

INTRODUCTION

Mushrooms are macro fungi, with distinct fruiting body, can either be hypogenous or epigenous (Chang and Miles, 1992). Economic importance and nutritional importance of mushrooms were increased as edible food this may be due to its high protein and fiber content and rich in antioxidants which play a major role in human health and nutrition. *Pleurotus* spp. belongs to Oyster mushrooms are nowadays very popular (Adejoye *et al*, 2006). Oyster mushrooms can be cultivated with agricultural and agro-industrial wastes as the substrates which is efficient and socio-economically reliable technology for converting wastes materials into a valuable protein-rich food and a cash crop of commercial interest. One of the most attractive feature of oyster mushrooms is that they can utilize a large variety of agricultural waste products and transform the lignocelluloses biomass into high-quality food, flavor and nutritive value (Dehariya and Vyas, 2013). It is important to dispose agricultural waste in a green way, which is environmentally friendly in this era of climate change. Annually about 140.8 MT of residues are available as surplus in India, out of which about 92.8 MT residues are burnt annually in the field with the intent of making the field free from straw and stubble after the harvest of the crop However, in case of rice and wheat fields, if combine harvesters are used for harvesting almost all the residues are left out in the field, resulting in in-situ burning (Gowda and Manvi, 2019). Mushroom cultivation is one of the most commercially important steps towards diversification of agriculture (Singh *et al.*, 2017). Microbial technology can help in large-scale recycling of agro waste as an alternative way to use agrowaste and organic material in mushroom production (Khare *et al.* 2010). Thus, the present investigations were carried out to evaluate the nutrient contents, phytochemicals and yield of wild and cultivated isolate of *Pleurotus djamor.*

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MATERIALS AND METHODS

Collection of materials

Pure Culture of *Pleurotus djamor* (oyster mushroom) was procured from University of Horticulture and Forestry Nauni, Solan and wild isolate was collected from the natural habitat, dead tree stumps from the forest of Dharamshala, Himachal Pradesh, India.

Identification of Mushroom

The collected mushroom was identified with the help of morphological characters of the colour, size, shape, nature of pileus, stipe and gills and molecular studies.

Maintenance of Pure culture and spawn preparation

A small piece of inner tissue of the fresh pileus was aseptically removed using a sterile forceps. Then, it was immediately placed on the surface of potato dextrose agar (PDA) plates and the plates were incubated at ambient temperature, from 20-24°C for 5-6 days. The pure culture obtained was maintained on PDA slants at 4±1°C in a refrigerator and subcultured at regular intervals for further studies. The spawn was prepared on wheat grains following standard protocol.

Molecular approach to identify the wild Isolate: Isolation of Genomic DNA

Total genomic DNA was extracted by following the methodology described by Vankan *et al*. (1991) with certain modifications.5.8S rRNA gene was amplified through polymerase chain reaction (PCR) using ITS 1 and ITS 4 primers. After amplification 5 µl aliquot of each resultant PCR product was mixed with 5 µl of bromophenol blue dye and electrophoresed on 1.5% agarose gel. PCR products were resolved by running at 50 volts. The gels were visualized under UV light and photographed using gel documentation system (Alphalmager® EP) with Alphalmager View software programme.

r RNA gene sequencing

PCR product was sent to Xcelris Genomics, Ahmadabad for sequencing ITS sequences obtained were compared with other reported *Pleurotus* ITS nucleotide sequences in GenBank database using nucleotide BLAST (Basic Local Alignment Search Tool) search (accessible through NCBI (National Centre for Biotechnology Information).

Evaluation of substrates for sporophore production

The experiment related to production of sporophores was conducted at college of Horticuture and Forestry Neri Hamirpur, Dr Y.S. Parmar University of Horticulture and Forestry Nauni during 2017-18. Dry paddy straw, wheat straw, maize stalks and Soybean straw substrates were evaluated for the cultivation of *P. djamor*. The selected substrates were chopped into 5 cm long pieces and transferred to gunny bags. The bags were soaked in clean tap water for 12 h. The excess water was drained out and the pre-s oaked substrates were sterilized for 30 min at 15 psi pressure. After cooling the substrates, the spawn of *P. djamor* was inoculated layer by layer until it covered the bags. The inoculated bags were incubated in dark for 12-14 days at a humidity range 80-90% and the temperature between 25 to 30°C for mycelial growth. When mycelial growth had covered the whole substrate in the bags, the bags were hanged and exposed to light of 2000-3000 lux units intensity for development of fruiting bodies.

Nutrtional studies

Standard biochemical techniques were employed for determining the nutritional composition of two *Pleurotus* species (AOAC, 1984; Lowry *et al*, 1951).

Preliminary phytochemical screening

Phytochemical screening experiment was conducted at Shoolini University of Biotechnology and Management Sciences (Himachal Pradesh). The extraction method of Kamra and Bhatt (2012) with certain modifications was used. The dried fruiting bodies were ground to a fine powder using a blender. For preparing the extracts, methanol and aqueous (50:50, v/v) were used as solvents to obtain the bioactive compounds from the wild and cultivated edible mushroom. For every 1 gram of powder, 50 ml of solvent was used and was subjected to extraction. After the completion of extraction, the supernatant was filtered through Whatman filter paper 1. The freshlyprepared extracts were subjected to standard phytochemical analysis to ensure the presence of phytochemicals Harborne (1998); Adebayo and Ishola (2009).

Antibacterial assay

Antibacterial activities of both the isolates of were done by treated through disc diffusion assay given by Kirby-Bauer, 1966. The bacterial strains were used in this study were *Escherichia coli* and *Staphylococcus aureus.*

RESULTS AND DISCUSSION

Pileus colour of the wild isolate was pink The surface of pileus was smooth and wavy, stipe length was 0.5-1.0 cm. Spore print was white with spore size of 6.1-7.2 x 4.4-5.2 to 6.5-7.8 x 4.6-5.3µm. Based on these morphological characters the isolate was identified as *Pleurotus djamore.* Other researchers like Ravat and John (2016) and Bernardo *et al.* (2004) have also used morphological characters to identify *Pleurotus* species. To further confirm the identity of the isolate molecular studies were conducted.

Species differentiation can't be solved merely on the basis of classical taxonomy. The internal transcribed spacer (ITS) region due to highly variability are important for molecular systematic in order to distinguish the species or strain of fungi (Moncalvo *et al*, 1995). These ribosomal DNA (rDNA) sequences have been widely used to discriminate fungal taxa at the family, generic and sub-generic levels. In present study, polymerase chain reaction (PCR) amplification of ITS-rDNA region of wild *Pleurotus* isolate using ITS1and ITS4 primers resulted in amplicon size products of approximately 595 basepairs. Sequencing and sequence analysis using nBLAST revealed 99% sequence similarity with *Pleurotus djamore* Accession No. KX904522.1Consensus sequence of (595 bp). Dung *et al,* (2012) studied the molecular identification methods for identification of two strains of white oyster mushroom.

Table 1: Comparative performance of different substrates on spawn run and yields of two isolates of *Pleurotus djamore.*

Substrate	*P. djamore* (cultivated)		*P. djamore* (wild)	
	Spawn run(Days)	Yield (g/kg wet substrate)	Spawn run (Days)	Yield (g/kg wet substrate)
Paddy straw	19±0	142.33±2.84	17±0	160.33±2.09
Wheat straw	19±0	114.66±3.71	*18*	110.66±4.02
Soybean straw	20±0	98.33±3.38	19±0	100.33±2.06
Sawdust	21±0	88.33±3.38	20±0	74.33±4.11

Consensus sequence of (595 bp)

tttgaagttg ttttgctggt ctctagggac attgtgcacg cttcattagt ttccacttca taccctgtg caccttgat agatttcggt ttgggttatc ctttggtttt ttttyytwaw tkraaaggcc tttggtttcc ttaaaacgac ttctatacta taccacacac caaatgtatg ttttataatg aatggtttat aatgacaagg ccatgagcct tataaactta atacaacttt caacaacgga tctcttggct ctcgcatcga tgaagaacgc agcgaaatgc gataagtaat

gtgaattgca gaattcagtg aatcatcgaa tctttgaacg caccttgcgc cctttggtat tccgaagggc atgcctgttt gagtgtcatt aaattctcaa actctatgac tttattgttg tagctgtttg gattgttggg ggttgctggc ttctttcttt gaagtcggct cctcttaaat gcattagcgg gactttgttg cctctgcgca tagtgtgata attatctacg ctagacgcat gcaattctta tattgtccag ctttctaatc gtctcaaggg acaattactt tgaca

The results presented in Table 1 indicates that response of both the *Pleurotus* isolates examined to different kind of substrates was similar. Both the isolates of *P djamore* cultivated and wild differed amongst one another with respect to the level of yields. The highest yields, irrespective of the kind of isolate was recorded on paddy straw followed by wheat straw and lowest yield was recorded on saw dust. Yield of wild isolate was more (160.33±2.0 g/kg wet substrate) on paddy straw as compared to cultivated isolate. The result of present studies are in agreement with Sharma *et al.* (2019) and Tupatkar and Jadhao (2006) who reported good yield of *P.djamore, P.ostreatus and Pleurotus florida* and *P sajor caju* on Paddy straw and soybean straw. Hossain (2017) reported maximum yield and biological efficiency of *P. sajor-caju w*ith Paddy straw. Superiority of paddy straw over other substrates in cultivation of *P. sajorcaju* with respect to yield and biological efficiency has also penned earlier by Pala *et al.,* 2012; Sharma *et al.,* 2019.

Table 2: Nutritional composition of *Pleurotus djamore* (wild and cultivated) isolate cultivated on paddy straw.

Nurtients (%)	*Pleurotus djamore* (cultivated)	*Pleurotus djamore* (wild)
Moisture*	89.6±0.05	90.9±0.99
Protein**	28.7±1.67	32.3±0.50
Carbohydrate**	50.3±0.52	40.9±0.31
Crude fat**	3.8±0.70	1.8±0.21
Crude fibre**	14.9±0.83	11.4±0.38
Ash**	9.8±0.09	8.0±0.41

Values expressed as mean ± standard deviation of three determinations (n=3); *denotes value on wet weight basis; **denotes values on dry weight basis.

Table 3: Qualitative analysis of phytochemicals in *Pleurotus djamore* isolates.

Phytochemical	Test	Wild isolate		Cultivated Isolate	
		Methanol	Aqueous	Methanol	Aqueous
Alkaloids	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Dragendroff's test	-	+	-	+
Carbohydtrate	Molisch's test	+	+	+	+
	Fehling's test	-	-	-	-
	Benedict's test	+	+	+	+
	Barfoed's test	+	+	+	+
Glycosides	Legal's test	+	+	+	+
	Keller-killiani test	+	-	+	-
Protein and amino acids	Millon's test	+	+	+	+
	Biurret test	+	+	+	+
Flavonoids	Shinoda test	-	-	-	-
	Alkaline reagent test	+	+	+	+
Phytosterols	Llibermaan-Burchard'test	-	-	-	-
Triterpenoids	Libermaan-Burchard'test	+	+	+	+
	Salkowski test	+	+	+	+
Phenolic compounds	FeCl3 test	+	+	+	+
tannins	Lead acetate test	+	+	+	+
Saponins	Frothing test	+	+	+	+
	Emulsion test	+	+	+	+
Anthraquinones	NH4OH test	-	-	-	-

Key: "+" denotes present "-" denotes absent.

Nutritive values of *Pleurotus djamore*:

Nutritional parameters such as moisture, protein, carbohydrate, fat, fibre and ash were measured and the results were tabulated (Table 2). Except moisture and protein content, other nutritional parameters such as carbohydrate, fat, fiber were found to be more in cultivated isolate of *P djamore* than wild isolate. The moisture contents in mushroom fruiting bodies of wild and cultivated isolate were found to be 90.9±0.99% and 89.6±0.05%, respectively. The protein content was 32.3±0.50g^{-1} in wild isolate and 28.7±1.67g^{-1} in cultivated isolate. The carbohydrates and fibre contents of wild isolate were 40.9±0.31g^{-1} and 11.4±0.38g^{-1}, respectively and in cultivated isolate 50.3±0.52g^{-1} and 14.9±0.83g^{-1}, respectively. Syed *et al*. (2009) cultivated *Pleurotus florida* on different agro-wastes and reported that soybean straw showed significantly highest yield with maximum crude protein of 23.50%. Yadav, 2015 reported maximum protein and carbohydrate content in *P. sajor-caju* and *P. florida* grown on wheat straw. Oyenuga, (1968) reported that mushrooms are the rich source of protein than most commonly consumed vegetables, whilst their protein content is lower than that found in eggs, meat and fish; however it is adequate to be used as a substitute in the diet of the general public. Lindequest *et al.* (2005) stated that the nutritional and chemical compositions of mushroom are responsible for their medicinal values.

Qualitative analysis of phytochemicals

The phytochemicals of both the isolates of *Pleurotus* were qualitatively analyzed and the results were presented in Table 3. The phytochemical analysis of *Pleurotus djamore* isolates showed the presence of major phytoconstituents like, alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids. This result is in agreement to the previous findings reported by Okwulehie and Ogoke (2013).

Among the two solvents used for extraction, methanol extract showed more number of phytoconstituents as compared to aqueous. Thus results suggested that extraction by methanol gave highest phenolic content as compared to the other solvents thus proving methanol to be a suitable solvent for the extraction of phenolic compounds. The reason could be that methanol has the ability to inhibit the reaction of polyphenol oxidase that cause the oxidation of phenolic and also evaporate with ease as compared to other solvents (Almey, 2010).

The antibacterial activity of wild and cultivated isolate of *P djamore* against Gram positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were evaluated by using standard zone of inhibition (Table 4). Vancomycin in Gram-positive bacteria and amicosin in Gram-negative bacteria were used as a standard antibiotic. Our results showed that methanol extract of wild isolate of *P djamore* showed maximum zone inhibition on (Gram-positive bacteria) *S. aureus* 8.6±0.2 when compared with aqueous extract that showed 6.6±0.2 mm zone of inhibitions. Whereas, methanol extract and aqueous extract of cultivated isolate of *P djamore* showed maximum inhibitory zone as 8.2±0.2 mm and 3.2±0.2 mm for *S. aureus* respectively. Aqueous extract showed minimum zone of inhibition irrespective, of the isolate and micro-organism. These observations are in accordance with the findings of Nehra *et al,* (2012) who reported the antimicrobial potential of *P. ostreatus* and found that "organic solvents consistently displayed better antimicrobial activity than that of the aqueous extract.

Table 4: Antimicrobial activity of *Pleurotus djamore* by disc diffusion method after 24 hours.

No.	Extract	Zone of inhibition (mm)			
		P.djamore (cultivated)		*P. djamore* (wild)	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
1	Methanol	6.2±0.2	8.2±0.2	6.6±0.2	8.6±0.2
2	Aqueous	1.4±0.4	2±0.2	1.8±0.4	2.4±0.4
3	Control	7.2±0.2	8.5±0.3	6.8±0.4	9.0±0.2

CONCLUSION

The present study reveals that Pleurotus isolate collected from natural habitat was identified as *P. djamor* on the basis of molecular studies. It could be a source of phytochemicals and antibacterial agent that may have beneficial health effects on humans. It possesses good amount of quality proteins. However, further research is required. The findings of the present paper warrant further research aiming to isolate and identify the specific compounds responsible for antibacterial activities.

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