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Original Article

Pharmacognostic, Chemical Characterization Studies on Oyster Mushroom (*Pleurotus ostreatus*)

Prasad Vijay Kadam*, Damini Kallyanrao Giram, Sameer Khamroddin Shaikh, Kavita Nilesh Yadav, Abhijit Sampatrao Karanje, Manohar Janardhan Patil

Department of Pharmacognosy, Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, INDIA.

ABSTRACT

Aim: The purpose of this study is to determine the Pharmacognostic, phytochemical and physicochemical standardization of *Pleurotus ostreatus*. **Materials and Methods:** The disease-free oyster mushroom fruiting body was collected during the rainy season from Himgiree Hi-Tech Agro (Mushroom Division) Kalyani Nagar Pune. Their identity and authentication were done. Macromorphology, cytomorphology, powder microscopy, micrometry, physicochemical evaluation, and phytochemical screening were done according to the WHO guidelines and standard methods. **Results:** Macromorphology of mushroom structure has a broad, oyster or fan-shaped cap, spanning 2-35 cm. yellowish grey colour, chocolaty odor, and significant taste. Cytomorphology of mushroom shows different cells such as aciculate, sphaerodunculate, lanceolate thick-walled, utriform, cornuate, Subcapitate, mucronate, cornuate, clavate 4-sterigmate basidium and in powder microscopy nodulose, isodiametric, snout like pore, spiny, bullet shaped, cuboid. The preliminary phytochemical analysis of the mushroom shows the occurrence of active compounds such as flavonoid, tannin, glycoside, coumarin, phenols, steroid, terpenoids protein and amino acid. **Conclusion:** The results obtained in this study the Standardization of phytochemical, physico-chemical is a fundamental dimension for sample identification, quality, and purity of oyster mushroom *(Pleurotus ostreatus)*.

Keywords: Cytomorphology, Oyster mushroom, Pharmacognostic study, Standardization.

Correspondence:
Dr. Prasad Vijay Kadam
Associate Professor, Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune-411033, Maharashtra, INDIA.
Email: kadamprasadv@gmail.com

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INTRODUCTION

The oyster mushroom *(Pleurotus ostreatus)* or Hiratake is a common mushroom that can be eaten. This was first grown in Germany. It may also be used to achieve mycoremediation.[1] These mushroom's name is originating from Latin (*Pleurotus* - sideways, oyster - the shape of the cap). The English common name is an oyster mushroom. It is a macro fungus of the phylum Basidiomycota, family Pleurotaceae. It is also called 'Dhingri' or abalone. A mushroom is defined as the fleshy spore carrying the fructification of the mushroom which may be epigenic or hypogen once formed and big enough to be seen to the naked eye and plucked by hand.[2] The mushroom has a broad, oyster, or fan-shaped cap. The gills of the mushroom are cream to white. The spore is white to lilac-grey. It is a good source of food as well as medicine. Several species of mushrooms are present all over the world. A small number of species are known to be poisonous, but the oyster mushroom has been popularly consumed by people due to its delicacy taste, texture, and high nutritional and medicinal value.[3]

They are a good source of vitamins and minerals and low in calories and fat. It is a good source of Beta-glucans. It is frequently provided as a nutritional component and with potential profitable value. It is considered a good source of Nutraceuticals and used in traditional medicine for a wide range of diseases because of its medicinal value.[4] The oyster mushroom contains primary metabolites like Carbohydrates, proteins, vitamins, dietary fibers, and much less cholesterol. It contains some secondary metabolites such as Ergothioneine, carotenoids, and Ascorbic acid. The phytochemical constituent of the mushroom includes flavonoids, phenols, tannins, saponins, polyphenols, terpenoids, and glycosides. It also contains minerals like sodium, calcium, magnesium, ferrous, zinc, copper, and manganese. These are good sources of protein. Globulin is the most abundant protein, prolamin, glutelin, and albumin were also present. It is used in cosmetic products for wrinkles, uneven tone, fine lines, and texture because it has potent antioxidant and anti-inflammatory activity.[2,5-7] They are a good source of prebiotics and contain short-chain sugars such as galactose, glucose, fructose, and N-acetylglucosamine. The bioactive components that are present in mushrooms contain peptides, polysaccharides, lipopolysaccharides, nucleosides, glycoproteins, lectins, lipids,

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and triterpenoids.[8] Methanolic extract of mushrooms shows the most potent free radical scavenging antioxidant activity.[9]

The presence of several bioactive compounds that exhibit great medicinal properties such as Immunomodulation,[10] Antiplasmodial,[11] Hypolipidemic,[12] Antioxidant,[13,14] Antimicrobial,[15] Antiatherogenic,[16] Anticancer,[17] Antihyperglycemic,[18] Atherosclerosis,[19] Antihypercholesterolemic,[20] Anti-inflammatory,[21] Antibacterial,[22] Antiviral, Anti-diabetic,[23] Inhibits HIV-1 reverse transcriptase, Eye health, Inhibition of protein synthesis, a proteolytic enzyme. The fruiting body of the mushroom can be used to remove waste products. Antifungal and antibacterial activities have been observed in the species. It is thought that extracts and isolated compounds were used as a defense mechanism against other organisms.[24] In mushrooms-glucans are present these β-glucans can be used as an active ingredient with UV light-protective and comforting properties after UV exposure for the cosmetic and pharmaceutical industries.[25]

One of the actual, specific, and well-organized methods for the characterization and estimation of herbal products is the Pharmacognostical study that includes some aspects such as microscopic, macroscopic, and phytochemical screening. Herbal drugs are easily susceptible to being substituted or adulterated with cheap quality substances. These adulterated substances decrease the therapeutic ability of herbal drugs.[26]

The present study is related with the standardization of medicinal mushroom *(Pleurotus ostreatus)* by using Pharmacognostic (Microscopy, Cytomorphology, Physical character) and Phytochemical study of the basidiocarp. All parameters were studied according to the WHO guidelines and Pharmacopoeial guidelines to standardize the Oyster mushroom.

MATERIALS AND METHODS

Materials

The Fruiting body of the Oyster mushroom *(Pleurotus ostreatus)* was collected during the rainy season from Himgiree Hi-Tech Agro (Mushroom Division), Kalyani Nagar, Pune. It was kept in a dry sterile plastic bag to reduce decomposition. Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune provided ethanol, methanol etc. (Figure 1).

Morphology and organoleptic evaluation of Mushroom

Several morphological traits, including mycelium growth, the height of the fruiting body, stipe length, stipe diameter, and pileus diameter, were used to identify the oyster mushroom.

Figure 1: Oyster mushroom (*Pleurotus ostreatus*).

Cytomorphology

Fruiting bodies of mushroom were hand sectioned transversely from the hymenium. Slice a very thin-cross-sliced section with a sharp diamond-edge razor blade. Photomicrographs were taken with a 10x digital microscope and were analyzed by Motic image plus 2.0 software.[27]

Powder microscopy

The powder of basidiocarps was studied microscopically and characters were explored as per the reported methods.[27]

Micrometry

Quantitative microscopy of the powder was performed to identify the size and dimension of the cellular structure of basidia, hymenophoral trama, and basidiospore. Photomicrographs were taken with a 10x digital microscope and were analyzed by Motic image plus 2.0 software.[27]

Physico-chemical Evaluation

The physico-chemical parameters such as ash values, extractive value, moisture content, swelling index were determined as reported by the official method of the WHO guidelines on quality control methods for medicinal plant material. [28]

Extraction

Fresh oyster mushroom fruiting bodies were blended into a coarse powder after being dried in a hot air oven from 35°C to 40°C. 300 gm of dried mushroom powder was extracted with 4 L of Ethanol by cold maceration technique keeping it at room temperature. The solvent was changed two times a week till the solvent coming from it becomes colorless. Then filter the extract using the Whatman filter. Make the extract solvent-free by using a rotary vacuum evaporator keeping the temperature in the range of 30°C-40°C. A Dark brown color residue was obtained having a characteristic odour. The further remaining solvent is evaporated to dryness by using a hot plate. The extracts were stored in an air-tight container for further use (Figure 2).

Figure 2: Ethanolic extract of Oyster mushroom.

Preliminary phytochemical screening of Mushroom

Qualitative Analysis

The qualitative analysis was done with the standard procedure described in Phytochemical methods. The test was carried out by using standard conventional protocols.[28]

The usual method outlined in phytochemical procedures was used to conduct the qualitative analysis. For the purpose of identifying the numerous phytoconstituents found in the powdered crude medication, qualitative tests were conducted.[28]

Quantitative Analysis

Total flavonoid content determination

The total flavonoid compounds present in the extracts were determined by using the aluminum chloride colorimetric method Evi Sulastri *et al*. 2018.[29]

Antioxidant activity by DPPH assay

The antioxidant activity of ethanolic extracts were determined by using DPPH (2,2 -diphenyl-1- picryl-hydrazyl) radicals assay as described by Arbaayah HH *et al*. 2013.[30]

Inhibition of DPPH radical (%) = $100 \times (A_{control} - A_{sample})/A_{control}$

Figure 3: Macro morphology of Oyster mushroom fruiting body.

Where, $A_{control}$ = Absorbance of the control solution (containing all reagents except the test extract) A_{sample} = Absorbance of the test extract.

Atomic Absorption Spectrophotometric Analysis

Atomic absorption spectrophotometric analysis were analyzed by using ethanolic extract as well as a lyophilized aqueous extract for Oyster mushroom (Pleurotus ostreatus) 500 mg extract powder + 10 mL conc nitric acid + 1mL conc. HCl + 1m) H_2O_2 (hydrogen peroxide) Heat on a hot plate till it comes to 3 to 4 mL. Cool and filter the solution in a 100 mL flask and make up "the volume by using 1% nitric acid. The analysis of samples was done according to metals. Lead, arsenic, calcium, sodium, zinc, magnesium, potassium, copper, iron, and manganese were analyzed by the flame system.

RESULTS

Macroscopy

The oyster mushroom has a broad, fan- or oyster-shaped cap that ranges in size from 2 to 25 cm. Natural specimens range in colour from white to grey. When the edge is young, it is smooth, slightly lobed, or somewhat wavy. Due to the stipe arrangement, the flesh is firm, white, and varies in thickness. The mushroom has a significant flavour, a chocolatey aroma, and a yellowish-grey colour, according to organoleptic examination. The results of macromorphology were illustrated in Figure 3, and Table 1 shows the morphology of the mushroom.

Cytomorphology

The transverse section of the lamellae fruiting body shows basidia, hymenophoral trama, and basidiospores (Figure 4) and the transverse section from the hymenium of the fruiting body shows Clavate, 4-sterigmate basidium, Sphaeropedunculate, Lanceolate thick-walled, Utriform, Aciculate, Mucronate, Cornuate (Antlered), Subcapitate (Figure 5).

Table 1: Macroscopical description of Oyster mushroom.

Characteristics	Observations
Colour	Yellowish grey
Odour	Chocolaty
Taste	Significant
Size	Spanning 2-25 cm
Shape	Cap shaped like an oyster shell
Type	Fungi

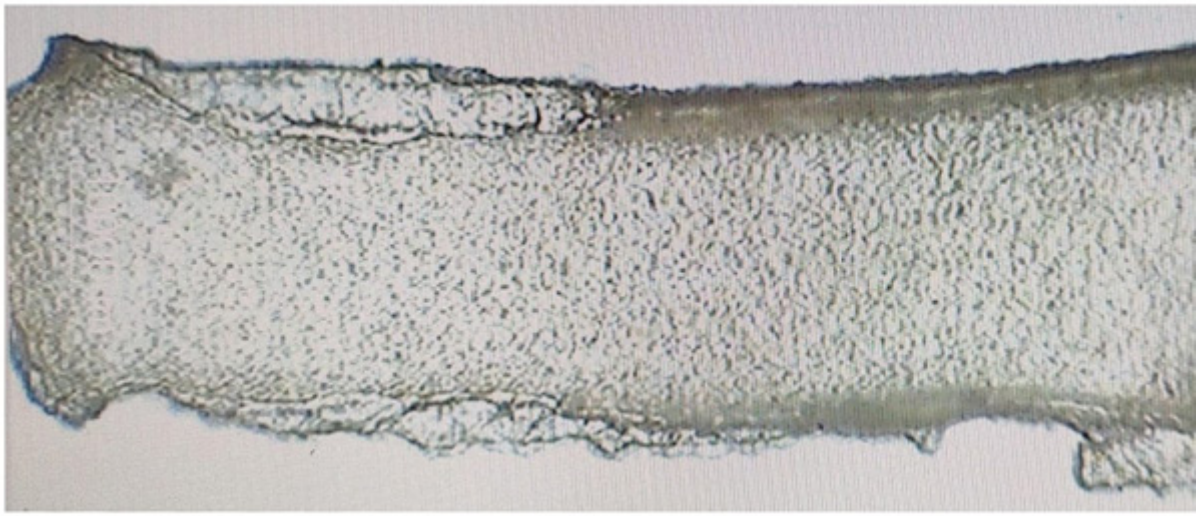

Figure 4: T. S of lamellae of fruiting body.

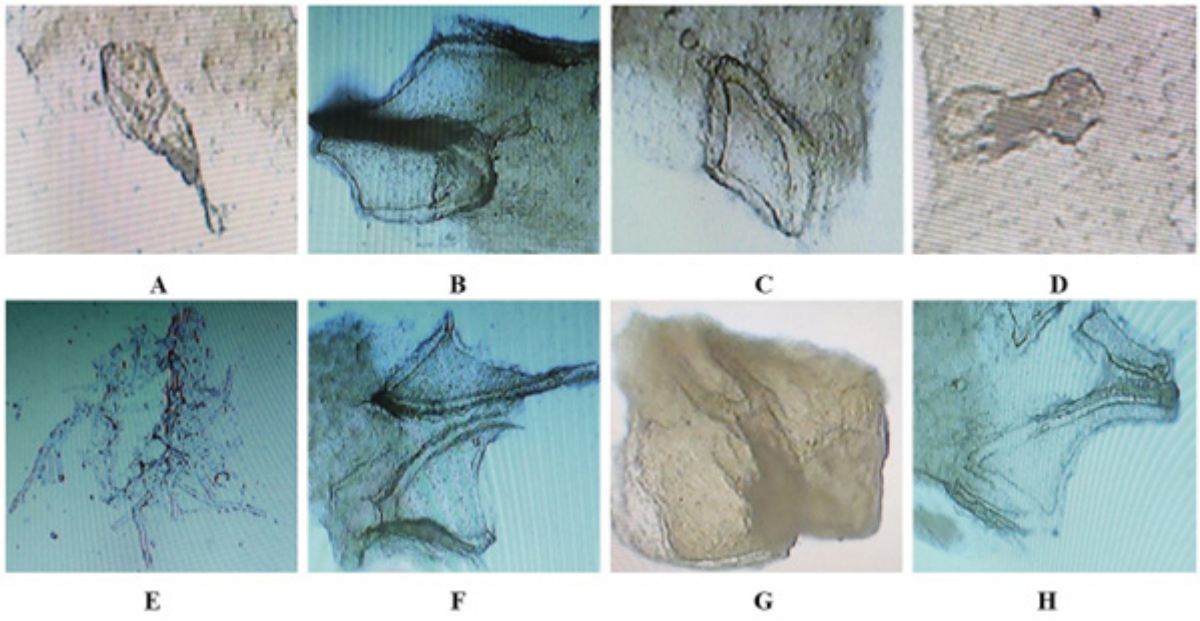

Figure 5: T. S of hymenium of fruiting body A – Clavate, 4-sterigmate basidium, B – Sphaeropedunculate, C – Lanceolate thick-walled, D – Utriform, E – Aciculate, F – Mucronate, G – Cornuate (Antlered), H – Subcapitate.

Powder characteristics

The powder of the mushroom fruiting body is grey-yellow, with an aromatic odour and strong taste. The microscopic investigation of the powder shows spiny, nodulose, striate, allantoid, perispore, ellipsoid, cuboid, reticulate, isodiametric, bullet-shaped, septate fusiform, echinate, perispore, snout-like pore is present (Figure 6).

Micrometry

The results of the micrometric characteristic of basidia, hymenopheral trama, and basidiospores are represented in Figure 7. The results of Quantitative microscopy of the hymenium of fruiting body are shown in Figure 8 and Table 2 and quantitative microscopy of the of fruiting body powder shows in Figure 9 and Table 3.

Physico-chemical Evaluation

The results of the physicochemical constant of raw material lie within the limit which is mentioned in Table 4.

Extraction of Oyster mushroom extracts

The nature of the extracts is dark brown and the yield is 8.9gm.

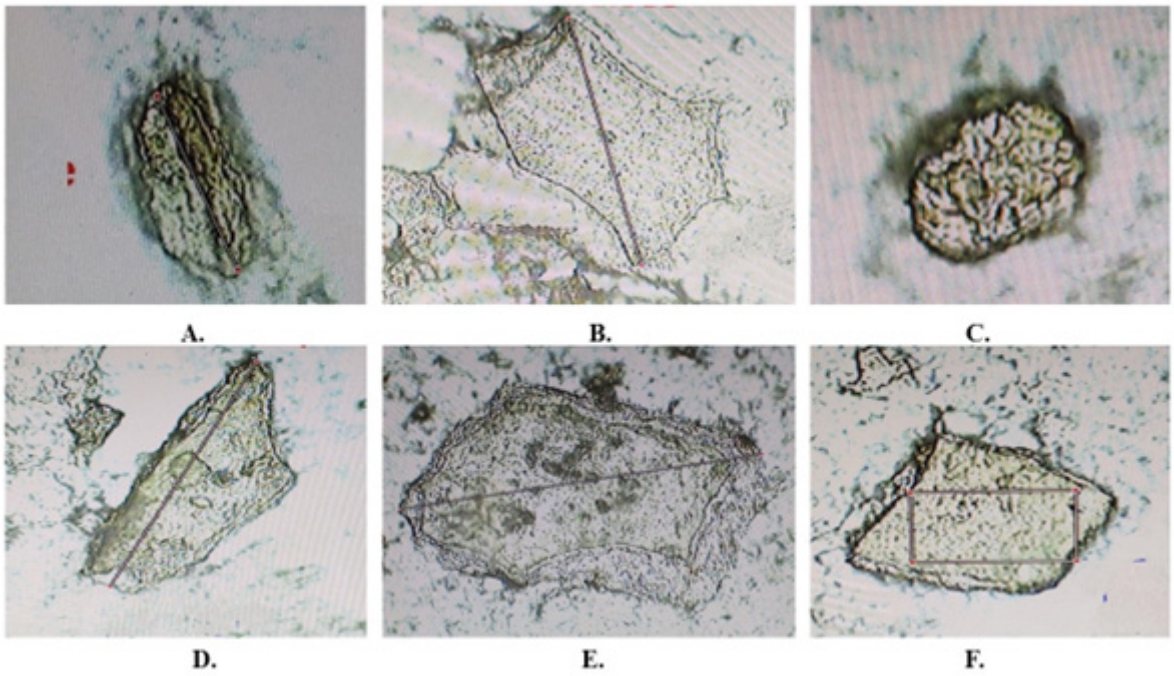

Figure 6: Powder microscopy of Oyster mushroom (*Pleurotus ostreatus*) fruiting body A: Snout-like pore; B: Nodulose; C: Spiny; D: Bullet-shaped; E: Isodiametric; F: Cuboid.

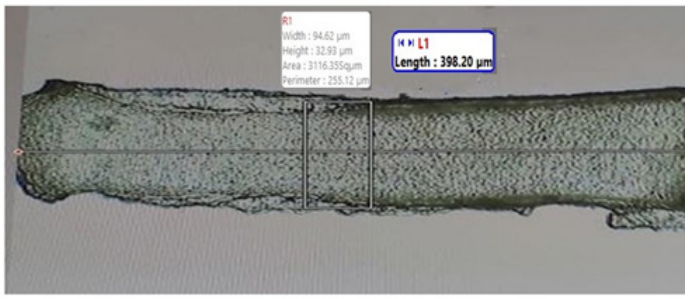

Figure 7: Quantitative microscopy of lamellae of fruiting body.

The transverse section of oyster mushrooms shows that spores are cylindric, smooth, with vacuoles and hyaline. Basidia are occasionally distinctive in shape, but they are clavate (shaped like inverted clubs). Between the basidia basidium-like cells that lack prongs and don't hold spores called basidioles. They are probably representing aborted basidia. The true spore-bearing basidia were almost large enough to be seen easily with an oil-immersion lens. Thus, the glossary entry for brachybasidioles is also called "pavement cells". Hymenopheral trama is regular or irregular. Cystidia are sterile cells found in mushrooms that crack up

Table 2: Quantitative microscopy of hymenium of fruiting body.

Sl. No.	Type of Cells	Length (µm)	Width (µm)	Heights (µm)
1.	Clavate, 4-sterigmate basidium	201.26 µm	58.87	27.65
2.	Sphaeropedunculate	215.80	105.38	41.10
3.	Lanceolate thick-walled	254. 08	144.63	29.89
4.	Utriform	109.32	17.44	35.87
5.	Aciculate	135.79	3.63	5.23
6.	Mucronate	158.59	79.94	23.92
7.	Cornuate (Antlered)	135.85	34.21	27.69
8.	Subcapitate	166.30	37.07	14.95

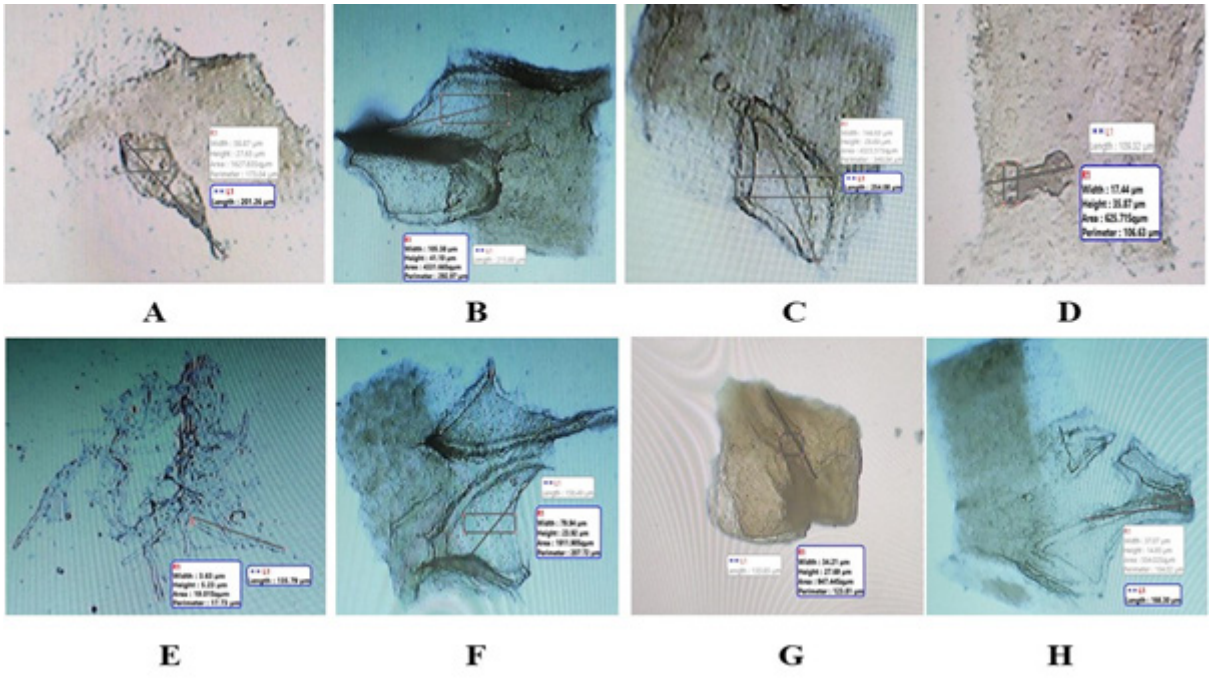

Figure 8: Quantitative microscopy of hymenium of fruiting body A. Clavate, 4-sterigmate basidium, B. Sphaeropedunculate, C. lanceolate thick-walled, D. utriform, E. Aciculate, F. Mucronate, G. Cornuate (Antlered), H. Subcapitate.

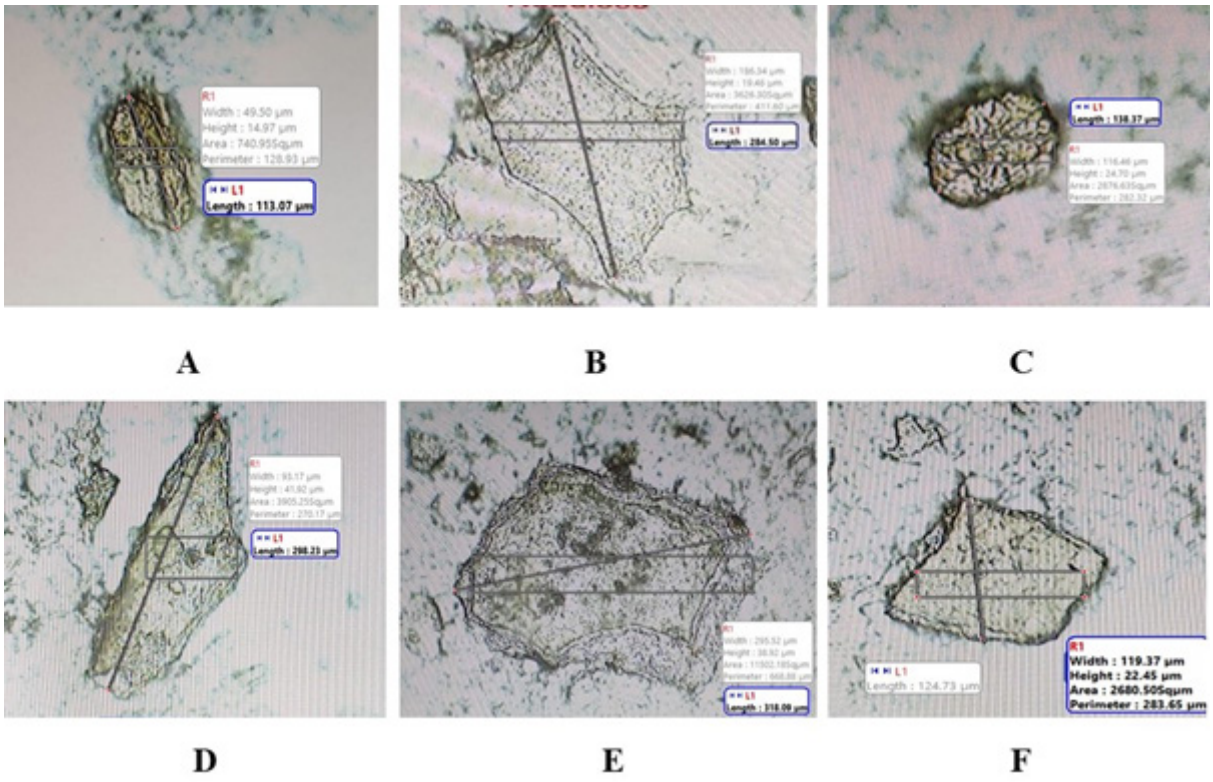

Figure 9: Quantitative microscopy of fruiting body powder *A-Snout like pore, B- Nodulose, C- Spiny, D-Bullet-shaped, E- Isodiametric, F. Cuboid.

Table 3: Quantitative microscopy of fruiting body powder.

Sl. No.	Type of Cells	Length in (um)	Width in (um)	Heights (um)
1.	Snout like pore	113.07	49.50	14.97
2.	Nodulose	284.50	186.43	19.46
3.	Spiny	138.37	116.46	2021
4.	Bullet-shaped	298.23	93.17	41.92
5.	Isodiametric	318.09	295.52	38.92
6.	Cuboid	124.73	119.37	22.45

Table 4: Physico-chemical Parameters

Sl. No.	Parameters (%w/w)	Observations
	Ash values	
1.	Total ash	7.4 ± 0.54
2.	Acid insoluble ash	0.25 ± 0.01
3.	Water-soluble ash	0.35 ± 0.02
4.	Sulphated ash	0.65 ± 0.05
	Extractive values	
5.	Water-soluble extractive value	31.5 ± 1.27
6.	Alcohol soluble extractive value	05 ± 0.28

Table 5: Preliminary phytochemical screening.

Sl. No.	Parameters	Observation
		Ethanol
1.	Carbohydrates	+
2.	Proteins	+
3.	Amino acids	+
4.	Steroids	+
5.	Flavonoids	+
6.	Alkaloids	-
7.	Tannins	+
8.	Phenolic compounds	+
9.	Saponins	+
10.	Anthraquinone glycosides	-
11.	Coumarin glycoside	+

*+ indicates the presence

Table 6: Total flavonoid.

Sl. No.	Analysis	Quantity
1.	Total flavonoid	0.25 ± 0.03

Preliminary Phytochemical Screening

Qualitative Analysis

In the preliminary phytochemical exploration of powdered fruiting body, achieved the presence of carbohydrates, proteins, flavonoids, steroids, saponin, tannins, phenols, and coumarin glycoside. The following Table 5 shows the qualitative analysis of oyster mushrooms.

Total flavonoid content of oyster mushrooms shown in Table 6 and Figure 10. Antioxidant Activity of the extracts of Diphenyl picryl hydrazyl: The result shows in Figure 11.

Atomic Absorption Spectrophotometric Analysis

AAS heavy metal data for ethanolic extract of Oyster mushroom lyophilized extract of Oyster mushrooms are shown in Table 7.

DISCUSSION

Various organoleptic characters, and microscopic, and phytochemical markers that have been created to aid in the classification and standardization of oyster mushrooms were revealed by the study. According to the macroscopic analysis, oyster mushrooms Pileus: oyster-shaped, 40-250 mm wide, thin to thick, meaty, radially fibrous, fungoid-smelling, and mildly flavourful. Lamellae: many, long-decurrent, smooth-edged, whitish to cream or light greyish in colour. Stipe: 10-20 X 10-25 mm, simple, typically lateral, numerous concrescent, surface longitudinally striate, whitish villose-pilose, solid context.[31] The adulterants and/or substituents present in the crude medication can be identified in this way with the use of optical microscopy, which is required for the quantitative identification of closely related molecules.[32]

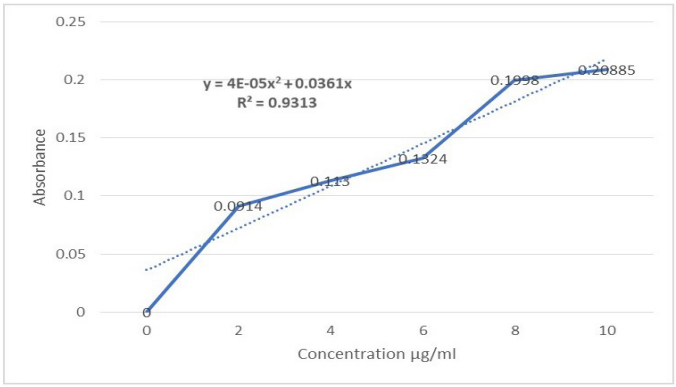

Figure 10: Calibration Curve of Quercetin Standard.

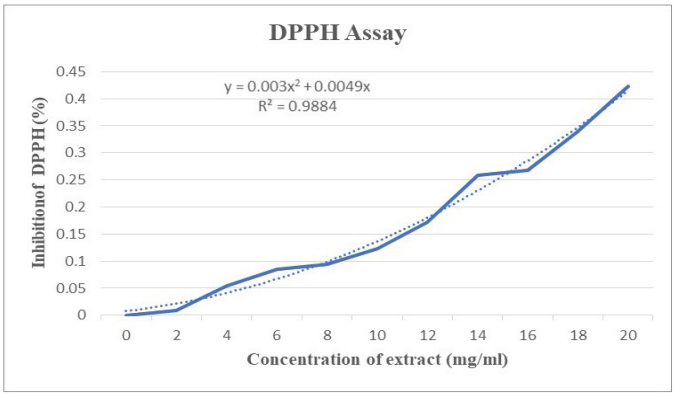

Figure 11: DPPH Antioxidant activity.

Table 7: AAS results for Ethanolic extract as well as aqueous extract of Oyster mushroom.

Sl. No.	Particular	Extracts	Observations
1.	Zinc (Zn)	EEOM	28.41
		AEOM	24.97
2.	Potassium (K)	EEOM	0.98
		AEOM	0.58
3.	Arsenic (As)	EEOM	0.11
		AEOM	0.11
4.	Calcium (CA)	EEOM	0.05
		AEOM	0.1
5.	Sodium (Na)	EEOM	0.91
		AEOM	0.67
6.	Magnesium (Mg)	EEOM	2.10
		AEOM	1.77
7.	Lead (Pb)	EEOM	0.65
		AEOM	0.65
8.	Iron (Fe)	EEOM	0.02
		AEOM	0.02
9.	Manganese (Mn)	EEOM	60.21
		AEOM	0.49
10.	Copper (Cu)	EEOM	1.06

between the basidia. Cystidia are thick-walled and enormous, long, and pointy. Pleurocystidia are found on the faces of the gills, whereas cheilocystidia are found on the edges of the gills.[6] In boletes, the pleurocystidia are found inside the walls of the tubes, whereas cheilocystidia are found on the edges of tubes. Pileipellis are flexuous and branched hyphae, septa with clamp connections. Cutis is a type of pileipellis. Ixocutis is one of the hyphae that are gelatinized as result shows mushrooms being viscid. In trichoderma, the hyphae appear perpendicular to the cap surface. Hymeniform pileipellis is club-shaped and appears perpendicular to the cap surface. These are immature basidia in a palisidia. Pileipellis is sometimes called cellular pileipellis.[27]

The measurements of different cells are necessary for the quantitative determination of closely interrelated species mixed with pure drugs as substituents and adulterants.[33] Thus, the substitutes or adulterants present in crude drugs can be distinguished by optical microscopy.

The Physical evaluation of crude drugs checks the quality and purity of drugs. The main reason to check the quality and purity of crude drugs biochemical variation in the drug, storage of

drug, substituent, and adulteration, and effect of treatment.[32] The results of the physico-chemical parameters of *Pleurotus ostreatus* powder as shown in Table 4. The inadequate drying approves contamination by bacteria and molds. The dryness of the drug is very important at which the moisture is removed. The determination of moisture content plays an important role in the method of preparation of a drug.[33] The total ash plays important role in the evaluation of the purity of the drug i.e., the absence or presence of foreign matter such as silica or metallic salts. The amounts of water-soluble ash were 0.35, acid insoluble ash 0.25. The alcohol-soluble extractive values indicated the presence of polar components like flavonoid, alkaloid, glycoside, phenol, and steroid.[34] The alcohol-soluble extractive value was found to be 5% w/w. The presence of acids, sugar, and inorganic compounds was indicated by the extractive values that are water-soluble.[33,34] The water-soluble extractive value was found to be 31.5% w/w. Which indicates the nature of the phytoconstituent present in the fruiting body.

The process of separating soluble material from an insoluble residue which may be liquid or solid by treatment with a solvent is known as extraction. Ethanol was used for extraction; ethanol extract showed more abundance of phytochemical constituents. These phytochemicals in mushrooms have been reported to have good phytochemical properties. For instance, higher amounts of flavonoids have been shown to protect against oxidative stress; while the presence of alkaloids has been reported to have a stimulating effect and potent antipyretic action. It can also act as a powerful anesthetic and pain reliever.[35]

The preliminary phytochemical Investigations of the powder drug of Oyster mushroom were performed which shows the occurrence of carbohydrates, proteins and Amino acids as primary metabolites and flavonoid, tannin, phenolic compounds, saponin, steroids, coumarin and anthraquinone glycosides as major secondary metabolites which revealed their potent therapeutic activity.[28]

The Total flavonoid assay was estimated to extract flavonoids, isoflavonoids, and neoflavonoids or collectively called bioflavonoids. These ketone-containing compounds will form acid-stable complexes with the C-4 keto group and either the C-3 and C-5 hydroxyl group of bioflavonoids. However, this method showed limitations to estimate flavonoids, the non-ketone group flavonoid. flavonoids responded poorly in the aluminum chloride colorimetric method flavonoid may act as antioxidants by breaking the radical chains into more stable products in liver microsomal membranes, with the ability to protect low-density lipoprotein or LDL from being demolished by heavy metals and macrophages and play an important role to provide instinctive protection against oxidative stress and side effects by its contribution with vitamins. Oyster mushroom samples showed higher total flavonoid content. The Mushroom extract showed positive antioxidant activity by fading the violet color of the DPPH solution to yellow and pale violet. The scavenging activities of radicals were in direct proportion with the concentrations of the extracts. As the concentration of the extract was increased, the scavenging activity towards DPPH radicals was also elevated. The results in this assay were presented as the concentration of extracts capable to inhibit 50% of radical solution (IC_{50} value) where the extract with the lowest IC_{50} value was the greatest antioxidant holder.[30]

Atomic Absorption spectroscopy exposes the presence of heavy metals. The Ethanolic, as well as the Lyophilized extract of *Pleurotus ostreatus*, shows the presence of manganese, potassium, arsenic, zinc, sodium, copper, lead, magnesium, mercury, calcium, cadmium, and ferrous. So, according to WHO guidelines, it is evidence that extracted compound is pure. The presence of the mineral elements in significant concentrations confers general well-being and wellness to the consumer and increases the Basal Metabolic Rate (BMR) of the aged and growing children. The Fe and Zn improve general metabolism, as well as Hb and RBC concentrations in humans. Zn and Se contribute to the development of the brain of infants and children. [35]

CONCLUSION

The present study highlights the knowledge of the identity and quality of the mushroom *Pleurotus ostreatus.* It is very difficult to recognize the desired material from dry powder, mushroom samples are only based on microscopic character. So, these microscopic features may act as a phytochemical preliminary carry regarding the mushroom's point out. The mushroom powder organoleptic characteristic was evaluated and revealed. Standardization is a fundamental dimension for sample identification, quality, and purity. Physico-chemical and Chemical analysis of the fruiting body accommodates the quality and purity of the mushroom. The present study was useful for further pharmacological and therapeutic assessment together with the standardization of mushrooms.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

EEOM: Ethanolic extract of Oyster mushroom, **AEOM:** Aqueous Extracts of Oyster mushroom.

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