Integrated Biochemical, Antioxidant, and Structural Characterization of *Pleurotus ostreatus* from the Moist Forest Regions of Karnataka, India

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***Abstract*—The present study focuses on the biochemical and structural characterization of *Pleurotus ostreatus* (Oyster mushroom) collected from the moist forest regions of Chitradurga and Davangere districts in Karnataka, India. The research aimed to explore its nutritional composition, protein estimation, antioxidant potential, and molecular profiling using advanced analytical techniques. Fresh mushroom samples were collected, processed, and extracted using ethyl acetate and chloroform solvents. The total nitrogen and protein content were determined through the Kjeldahl method, while the Biuret test was employed to differentiate protein and non-protein fractions. Antioxidant activity was evaluated using the DPPH free radical scavenging assay, which revealed significant antioxidant potential, particularly in the ethyl acetate extract. The thin-layer chromatography (TLC) analysis exhibited distinct R_f values, indicating the presence of various bioactive compounds. Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of hydroxyl, amide, and polysaccharide functional groups, signifying the richness of proteins, lipids, and β-glucans. Scanning electron microscopy (SEM) demonstrated the surface morphology and porosity differences among the extracts. The results confirm that *Pleurotus ostreatus* possesses valuable nutritional and antioxidant properties, making it a promising candidate for nutraceutical and pharmaceutical applications. This comprehensive investigation contributes to understanding the biochemical composition and health-promoting attributes of *Pleurotus ostreatus*, supporting its utilization as a natural source of bioactive compounds for functional food development.**

***Index Terms*—*Pleurotus ostreatus,* Antioxidant activity, FTIR spectroscopy, Thin-layer chromatography, Nutritional analysis, Bioactive compounds**

I. INTRODUCTION

Mushrooms belong to the *Basidiomycetes* group of macro fungi. Mushrooms can grow either above the soil (epigeous), or below the soil (hypogenous). Due to lack of chlorophyll in mushroom it cannot synthesize its own foods it depends on dead and decay as their saprophytes. Mushrooms are the choice ingredients of gourmet cuisine globally, encompassing a unique flavour, which works culinary wonders. A total of 2000 species of mushrooms exist, 25 of which are accepted as food and few are commercialized (Ogidi *et al*., 2021). Mushrooms are also known for their nutritional, organoleptic merits and medicinal properties. Their therapeutic qualities, although known much earlier, have recently been acknowledged and valued. The healing properties of mushrooms were known in Chinese traditional medicine even as much as thousands of years before and they are still being used today. Mushrooms abound in essential amino acids, minerals, proteins,

and biologically active polysaccharides. They are predominantly consumed in Asian countries, however, in recent years, *Pleurotus ostreatus*, *Boletus edulis*, *Lentinula edodes* (Shiitake), *Ganoderma lucidum* (Reishi), *Trametes versicolor*, *Grifola fronda* (Maitake), *Agaricus bisporus* and *Agaricus subrufescens*, have been widely popularized worldwide (Killedar *et al.,* 2024). The lignolytic characteristics of oyster mushrooms, a white-rot fungus, have garnered significant research attention. Oyster mushrooms are popular due to their simple cultivation techniques, high biological efficiency, and nutritional and medicinal benefits (Aditya *et al.,* 2024). Mushrooms exhibit a diverse array of morphological features that distinguish them within the fungal kingdom. The fruiting body, composed of a stipe and pileus, presents a variety of shapes, sizes, and textures. The hymenium, located on the underside of the pileus, may consist of gills, pores, or spines and serves as the spore-producing structure. The attachment and position of the stipe, as well as the presence of remnants of the universal and partial veils (volva and annulus, respectively), contribute to the morphological complexity of mushrooms. These characteristics are essential for taxonomic classification and reflect adaptations to diverse ecological niches. Mushroom species exhibit a remarkable degree of morphological variation. Fungal spores, essential for species dispersal, typically measure 5 to 15 micrometres in diameter. Notably, spore colour, structure, and ornamentation vary significantly among mushroom taxa. They are commonly found in temperate and tropical forests, occurring naturally on decomposing logs or occasionally on dried trunks of both deciduous and coniferous trees (Rashid *et al.,* 2017).

Pleurotus ostreatus, a fungus, has a high nutritional value due to its high content of amino acids, vitamin C, and fats. The mycelium contains 0.1-0.2g of fats, including oleic acid and linolenic acid, and compounds with hypocholesterolemic action. Lovastatin, an approved drug used in dyslipidemia treatment, is found in mature mushrooms' lamella. Variable amounts of lovastatin have been found in samples from Japan, Taiwan, and Korea. Ergothioneine, a compound accumulated in animal cells and tissues exposed to oxidative stress, is considered suitable for adjuvant treatment of strokes, neurodegeneration, and cardio-vascular diseases (Killedar and Ramalingappa. 2025). An active β-glucan called pleuran in *P. ostreatus* extracts, which has potential applications in treating cancer, infections, and immune system disorders. Pleuran is a branched polysaccharide with a backbone of β-D glucopyranosyl linked with (1→3) bonds and every fourth residue is substituted with a (1→6) D glucopyranosyl group. The mycelium of *P. ostreatus* contains high contents of mineral salts such as potassium, phosphorus, calcium, iron, copper, zinc, magnesium, and selenium. Zinc ions (ZnII) were released from *P. ostreatus* to artificial gastric juice, with zinc contents in artificial saliva, stomach, and intestinal juices ranging from 1.88 to 2.83 mg per 100 g DW (Mazlan *et al*., 2020). Zinc is essential for protein synthesis, insulin homeostasis, and acts as a cofactor of over 300 enzymes, including superoxide dismutase. Its beneficial effects include wound healing, mental performance, eye yellow macula protection, and antioxidant properties (Piska *et al*., 2017).

II. MATERIAL AND METHODS

Sample Collection:

The study conducted a wild mushroom survey and collection of mushrooms in Chitradurga and Davangere biosphere reserved areas, located in the moist forest region of Chitradurga, following the rainy season from June to July 2024. The collection sites were forests, University Campus, residential area, botanical garden, parks and nearby villages of those above-mentioned areas of moist region. The collection of samples made by following the Hailing. Systematic and periodical survey of different locations and other habitats were done in the moist region. Necessary materials and equipment such as data recording sheet, digital camera for photography, digging equipment, card board were arranged and collection of samples were usually made during day time a field characteristic of mushrooms was recorded in the data sheet. The different varieties of mushroom samples were collected from the different regions (Rumainul *et al.,* 2015).

Mushroom Spore Imprinting:

Mushrooms reproduce using spores instead of seeds. A single mushroom spore is only 4 to 20 microns in size, which means a human could not see it without a microscope However, there is a simple way to observe the spores of a mushroom-create an art print out of

billions of spores! The spores of a mushroom are located in a variety of places, depending on the species. Many mushroom spores are found underneath the cap inside gills or pores. When a mushroom is placed gill- or pore-side down, the spores fall onto the paper to produce a print, revealing the pattern and colour of the spores (Abd EL-Razek, *et al.,* 2020). Many other mushroom forms exist, so if you find a mushroom without gills or pores, it may be holding its spores in teeth, ridges, or some other system. Mushrooms were collected from various locations, without disturbing the other part of the mushroom. Mushroom stem was separated which is attached to the cap. Cap gills was placed side down on paper, and a little water or sucrose solution was sprayed on the cap which trigger spore production. A bowl or container was kept over the mushroom and kept it overnight, then carefully uncovered and removed the mushroom next morning, let dry (Mueller *et al*., 2004).

Crude Extraction Method:

The *Pleurotus sp* mushroom was collected, washed, grated, and dried under shade for 2-3 days. After drying, it was cut into small pieces and boiled in distilled water for 15-20 minutes. The extract was filtered through Whatman filter paper, and the sample was weighed and extracted using chloroform and ethyl acetate (Oyetayo, *et* al., 2013). The mixture was shaken daily and incubated for 3 days. Filtrates were obtained in separate glass bottles and evaporated using a rotary evaporator. A semi-solid form of *P. ostreatus* was obtained, with each crude extract leaving a weight of 3-4 grams. The process involved weighing and incubating the *Pleurotus* for several days (Ozdal *et al.,* 2019).

Determination of Nitrogen Level:

The total nitrogen level was determined using Kjeldhal method, 200 mg of mushroom stalk powder was mixed with15 mL of concentrated sulfuric acid (H_2SO_4) 98%, then 9 g of potassium sulfate ($KzSO_4$) and 1 g of copper sulfate (CuSO4,) were added to speed up the reaction by increasing the boiling point of temperature reaction. The mixture was heated (350-380°C) for 3 h. The reaction was left to stand until the sample cooled; 60 mL distilled water was added cautiously (Mohammed *et al*., 2024). Distillation: in a distillation apparatus, the digested sample was introduced into the distilling flask, then 200 mL of water and 50 mL of NaOH 50% were gradually added. A few drops (6-7) of Tashiro's indicator (100 mg methyl red + 50 mg methylene blue + 10.4 mL distilled water + 100 mL ethanol 96% were added to 50 mL of boric acid 4% (w/v), then the mixture was put into a trapping flask. All ammonia passed over into the boric acid by heating the distilling flask and when NH, reacted with boric acid, the solution turned from red violet to green due to colour change of the indicator from acid to basic medium. Approximately 200 mL of condensate were collected. The condenser was washed with distilled water.

Titration: the green solution of the trapping flask was subjected to titration with HCl 0.05 mol/L until it turned violet. The procedure was terminated when the coloration had remained stable for approximately 5 min. The total nitrogen in the sample was calculated with the volume and concentration of HCl needed, then the protein contents was estimated using the conversion factor (Ramalingappa *et al*., 2025).

Calculation:

N% = V X M of acid X 1.4007/m

Where M: normality of HCl

V: volume of HCl consumed in titration

m: weight of sample in grams.

Protein content was estimated using the conversion factor 4.38 multiplying by the total nitrogen. The nitrogen level was determined using Kjeldahl method. Nitrogen of protein and non-protein components was calculated by the difference between total nitrogen and nitrogen of the chitinous material (Boureghda *et al.,* 2021).

Investigation on the Elimination of Protein and Non-Protein Components:

The elimination of protein and non-protein components from was investigated according to Biuret test, Raw material: 1 g was mixed with alkaline copper sulfate reagent, First deproteinization step: 1 g of dried raw material was reacted with 30 mL of NaOH solution (1 M) for 2 h at 80 °C (second method). The obtained supernatant impurities were collected and mixed with copper sulfate solution 1%. Then, 1 g of the alkali-insoluble part was reacted with 100 mL of acetic acid 20 for 6 h at 95 °C. Second deproteinization step: the washed insoluble part obtained after acetic acid treatment was reacted with concentrated sodium hydroxide 50% for 3 h at 100 °C, ratio 1:30 (w/v). The supernatant obtained by centrifugation was mixed with

copper sulfate solution 1%. In each reaction of alkaline-copper sulfate, the appearance of a blue colour indicates the absence of protein whereas a violet colour demonstrates its presence (Killedar *et al.,* 2025).

Antioxidant Assay [DPPH Free radical scavenging activity]:

The 2, 2-Diphenyl-1 picrylhydrazyl (DPPH) method was used to measure the free radical scavenging activity. For DPPH solution, 1 mg of the reagent (DPPH) was dissolved in 50mL of solvent (methanol). 5mL DPPH solution was added into six different test tubes containing 5mg extract and 5mL methanol, separately. Samples were incubated at room temperature for 20 minutes. Blank was taken without extract. Optical density (OD) value was calculated by spectrophotometer at 517nm wavelength. Antioxidant potential was measured by the following mathematical equation (Dila and Geldimyrat., 2022).

% activity = (Absorbance (blank) – absorbance (sample) × 100/ Absorbance (blank).

Thin Layer Chromatography:

In 1958, Stahl demonstrated application of TLC in analysis. It is at present an important analytical tool for qualitative analysis of a number of natural products. Thin layer chromatography (TLC) is one of the simplest and least expensive methods for detecting natural product constituents because the method is easy to perform, is reproducible and requires little equipment. Silica gel TLC plates were frequently used for the qualitative and semi-qualitative determination of compounds. Slurry was made using silica G60 in distilled water. The slurry was then poured onto glass plates in a TLC plate maker. The plates were made to 1 mm thickness and allowed to dry for 1 days. Before use, the plates were activated at 1000C for 1 hour and then TLC plates were spotted with 15µl volumes of the concentrated crude extracts of *Pleurotus* and then suitable solvent system like Benzene: Ethanol: Acetone (6:2:2) has been used to run the sample then the TLC plates were observed for the spot or appearance of band. The R_f valve was calculated using the formula (Mohammed *et al.,* 2024).

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Fourier-Transform Infrared Spectroscopy (FTIR) analysis of polysaccharide:

Fourier-transform infrared (FTIR) spectroscopy is a promising method for characterizing biological samples by their chemical composition, providing qualitative and quantitative estimates of lipids, polysaccharides, nucleic acids, and proteins. It serves as a global "molecular fingerprint" for characterization, differentiation, and identification of microorganisms, including bacteria, yeast, filamentous fungi, and some mushrooms. FTIR spectroscopy was used to analyse the nutritional profile of king oyster strains, revealing the presence of various functional groups in mushrooms. Mushrooms are rich in carbohydrates, proteins, and low fats. FTIR allows for specific characterization of nucleic acids, proteins, sugars, lipids, starch, and macrostructure. This technique is sensitive to molecular structure dissimilarities (Khan *et al*., 2019). The FT-IR spectra was resolved utilizing a Fourier change infrared Spectrophotometer on instrument Bruker. Test was squeezed into pellets, and afterward exposed to FT-IR spectrophotometer in the range of 4000–400 cm−1. Effects was checked at temperature 25°C, diluted alkali, concentrated alkali, hot water, and high pressure (D'Souza and Kamat, 2017).

Scanning Electron Microscopy (SEM):

Scanning Electron Microscopy (SEM) reveals detailed microstructural features of mushrooms. The surfaces of the powder particle structures were observed using a scanning electron microscopy (SEM). Thoroughly mixed samples of mushroom powders were applied to conductive carbon tape and covered with a thin layer of carbon (10 nm) using spraying system. Images with different magnification were taken for each sample (Sowmya *et al*., 2024).

III. RESULTS

Sample Collection:

Varieties of mushroom samples were collected from various locations in Karnataka (Chitradurga and Davangere districts), including University Campus of Davangere, Jogimatti forest area, residential areas, parks, and nearby villages, were analysed for their richness (Figure 01).

Macrocybe sp

Agaricus sp

Agaricus sp

Termitomyces sp

Lepiota sp

Pleurotus sp

Agaricus sp

Lepiota sp

Megacollybia sp

Fig 01. Collection of Mushroom Sample from different places (Davangere and Chitradurga Districts) like University Campus, nearby villages, Jogimatti forest area, parks, and residential areas.

Mushroom Spore Imprinting:

Mushrooms were collected from various locations, separated, and sprayed with water or sucrose solution to trigger spore production. The cap gills were placed side down on paper, and a bowl was placed over the mushroom overnight. The next morning, the mushroom was carefully removed and dried as shown in the Figure 02.

Fig 02. Mushroom Spore Imprinting of the various mushroom collected from the different locations.

Crude Extraction Method:

Different types of mushrooms was collected but for further experiment Oyster Mushroom was washed and dried for 2-3 days, then cut into small pieces. The small pieces of mushroom were boiled in distilled water for 15-30mins then the extract was filtered through Whatman filter paper. Then added 50ml of ethyl acetate and chloroform to the extract, the mixture was incubated for 3 days. After incubation for 3 days the extract was used for the estimations and analytical techniques (Figure 03).

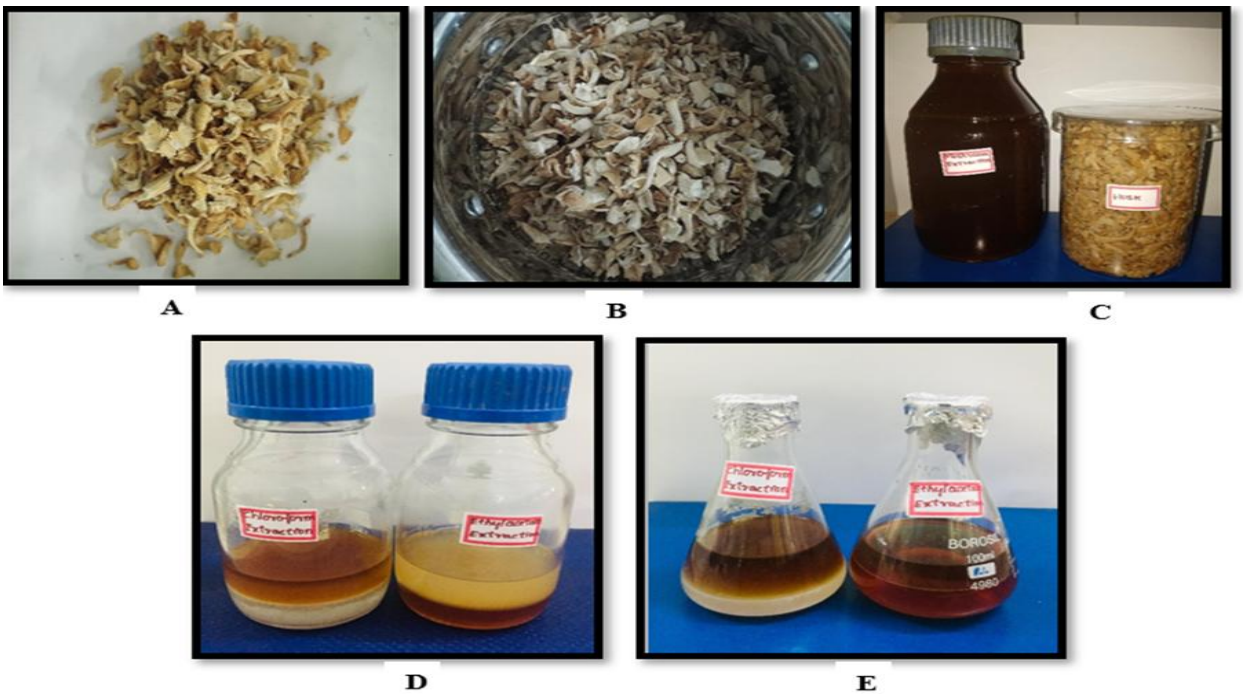

Fig 03. Crude Extraction Method: A: Cut the dry sample into small pieces, B: Boiled the mushroom for 15-20mins, C: Mushroom extract, D: Ethyl acetate and chloroform extract after incubation, and E: Crude ethyl acetate and chloroform extract.

Determination of Nitrogen Level:

Total nitrogen of the raw material was calculated using equation, the protein was estimated as (0.052%, 0.110%, and 0.048%) (Total nitrogen X conversion factor 4.38). The nitrogen content of the mushroom sample was calculated using Kjeldahl method. The nitrogen of protein and non-protein components was calculated to be 0.0126% in standard, 0.252% in Ethyl acetate extract and 0.011% in Chloroform extract (Figure 04 & Table.01).

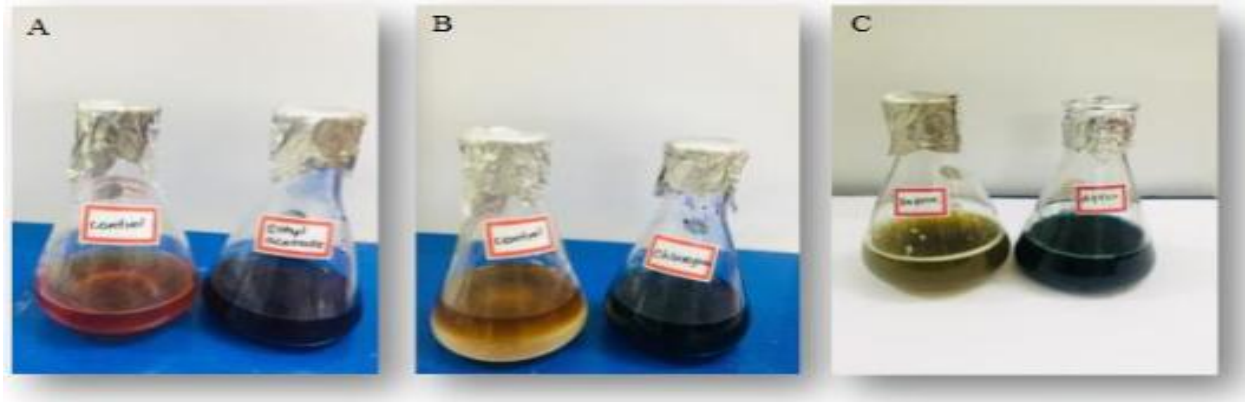

Fig 04. Determination of Nitrogen level of Oyster Mushroom using Kjeldahl method; A: Standard, B: Ethyl acetate, C: Chloroform

Table 01. Determination of Nitrogen level of the Oyster

Types	Raw materials (%)			Method of determination
	Standard	Ethyl acetate	Chloroform	
Total Nitrogen	0.0126	0.252	0.011	Kjeldhal method
Total Protein	0.0525	0.110	0.048	Total nitrogen X conversion factor (4.38)

Investigation on the Elimination of Protein and Non-Protein Components:

Biuret test was conducted to investigate the absence of protein in the sample. Ethyl alcohol extracted sample and alkali-soluble part has a light violet colour with alkaline copper sulfate reagent indicating the minimum amount of protein while the alkaline-soluble part and chloroform extract has a violet colour with alkaline copper sulfate reagent indicating the presence of protein. This result agrees with those of Kjeldahl method, which displays a decrease in total nitrogen content after the extraction (Figure 05).

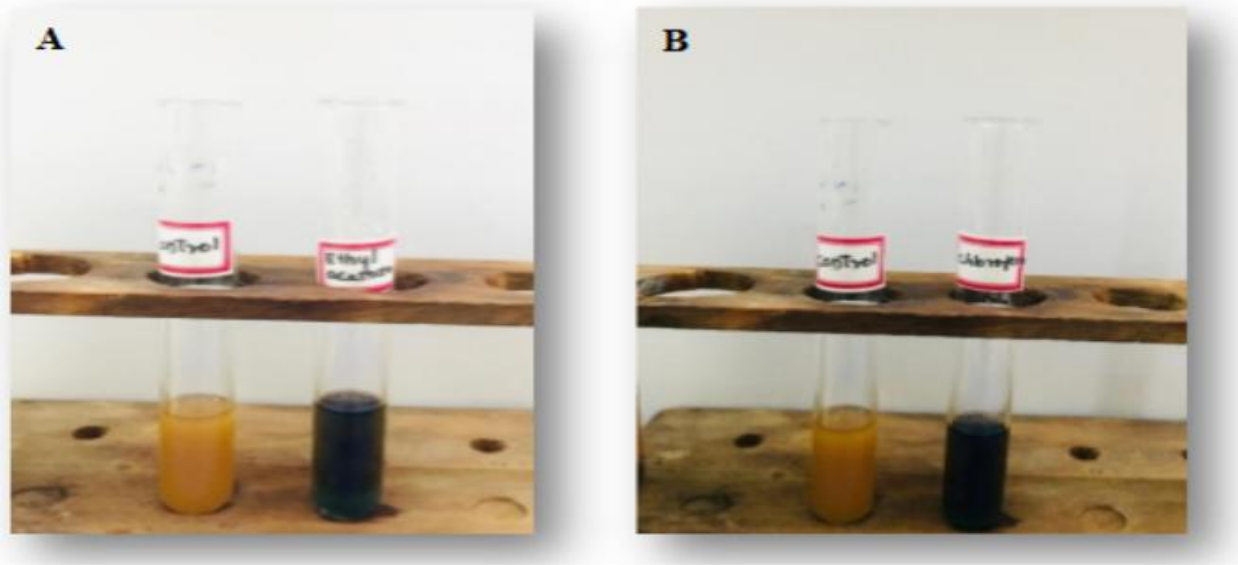

Fig 05. Determination of Protein and Non-protein level of oyster mushroom extract; A: Ethyl acetate B: Chloroform

Antioxidant assay:

DPPH Assay

The standard, ethyl acetate, and chloroform extract of mushroom was subjected to screening for their possible antioxidant activity. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in Figure 06. All concentration studied showed free radical scavenging activity. The 50% of inhibition value for standard extract seems to be fairly significant when compared to commonly used ethyl acetate and chloroform extract. The antioxidant capacity of mushroom extracts indicates the presence of phytochemicals, which are essential for health promotion. The standard extract showed the highest antioxidant activity, followed by ethyl acetate and chloroform extracts. These antioxidants can be used to enhance antioxidant defence through dietary supplementation to reduce oxidative stress levels. They also have medicinal properties and can be used to manage oxidative stress-induced diseases. Overall, these mushrooms can be beneficial for managing health issues. (Figure 06).

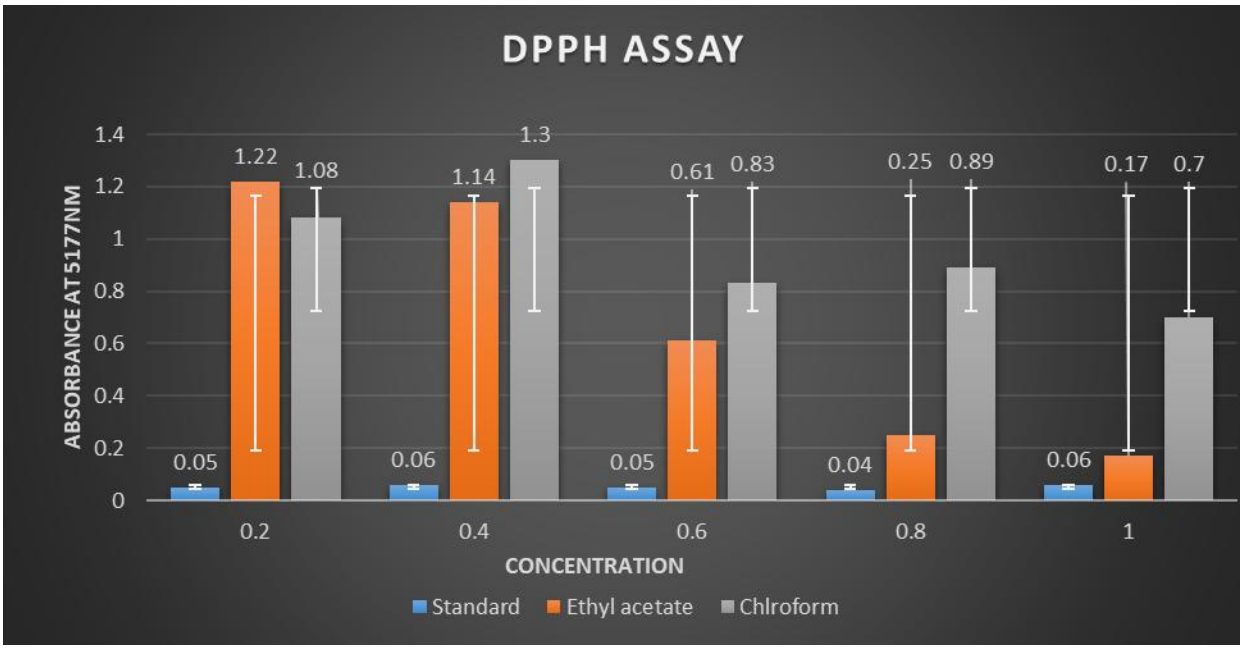

Fig 06. Antioxidant assay of different dilutions graph. A: ethyl acetate and B: chloroform extract of oyster mushroom

Thin Layer Chromatography:

Thin layer chromatographic profile yielded the different pattern of compound and as well as different R_f values. The extracted bioactive compounds were tested followed by calculate their R_f value by analysing the thin layer chromatographic technique with the different solvent systems. The standard extract yield showing the band and R_f value was 0.84 as shown in Figure 07. By using Benzene: Ethanol: Acetone (6:2:2) as a mobile solvent. Similar mobile solvents are used in Ethyl acetate and Chloroform extract, where chloroform extract shows similarity band with that of standard having the R_f value 0.92 as shown in Figure 09. But Ethyl acetate extract having the band and R_f value at 0.42 as shown in Figure 08.

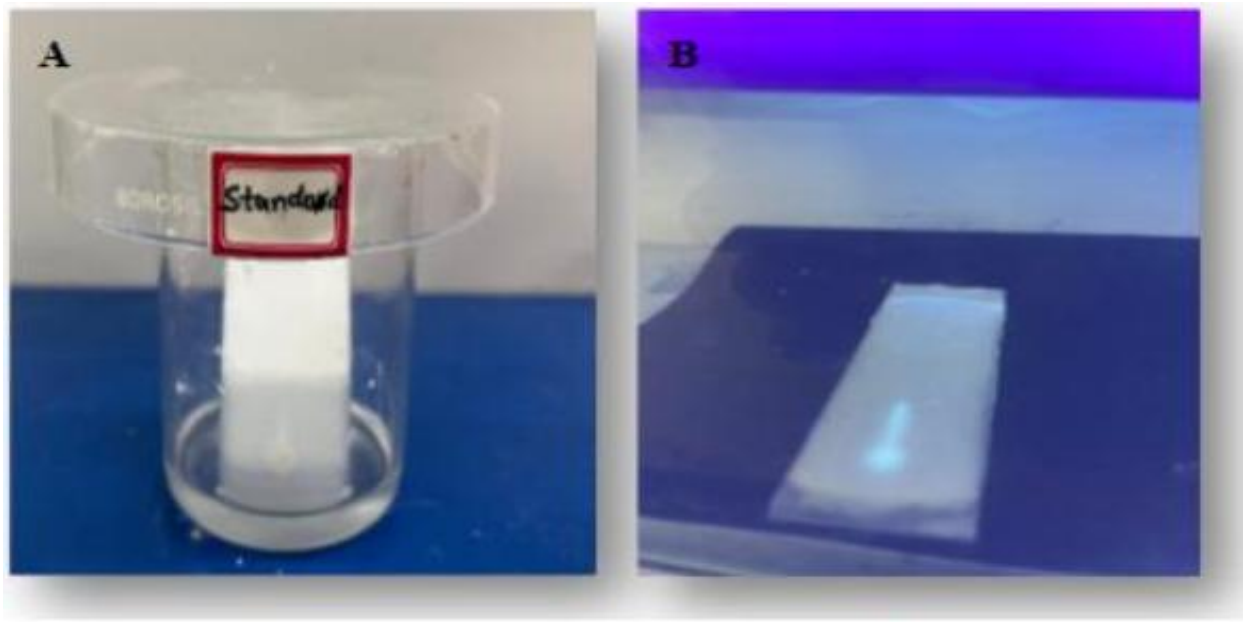

Fig 08. Thin Layer Chromatographic analysis of Standard: A. Loaded sample in mobile phase, B. TLC plate under UV light

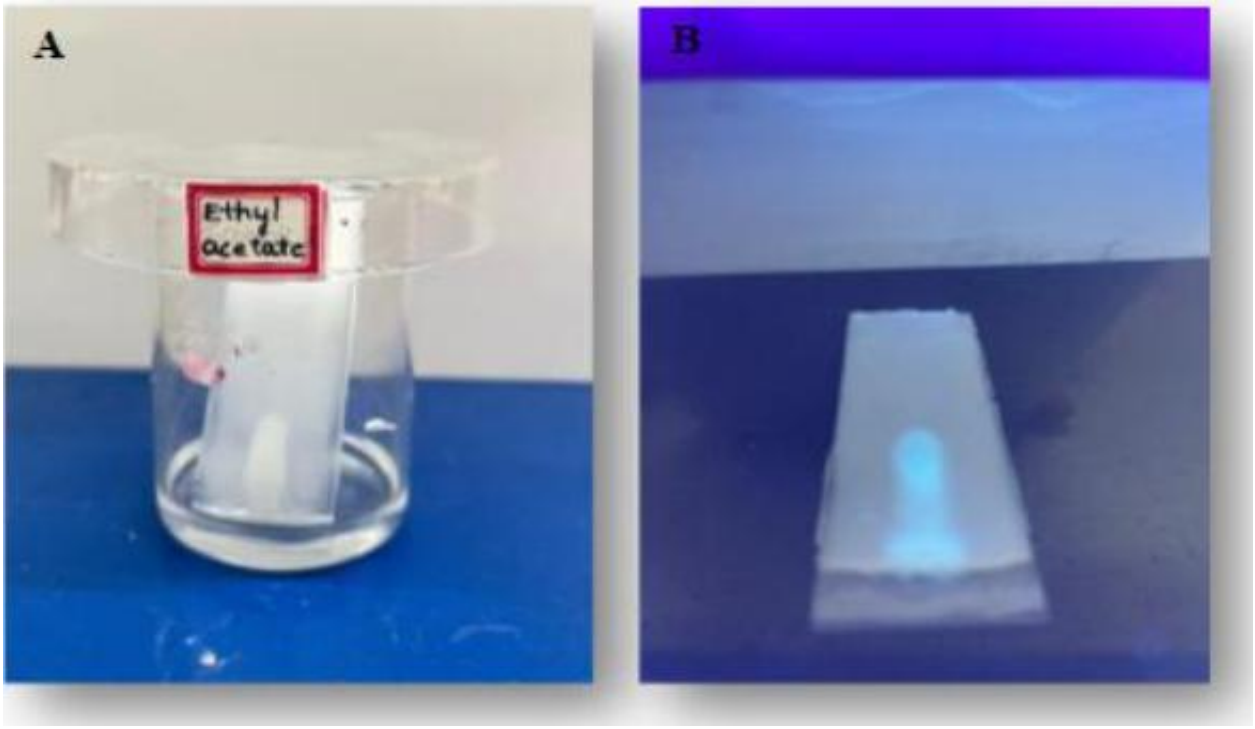

Fig 09. Thin Layer Chromatographic analysis of Ethyl acetate: A. Loaded sample in mobile phase, B. TLC plate under UV light

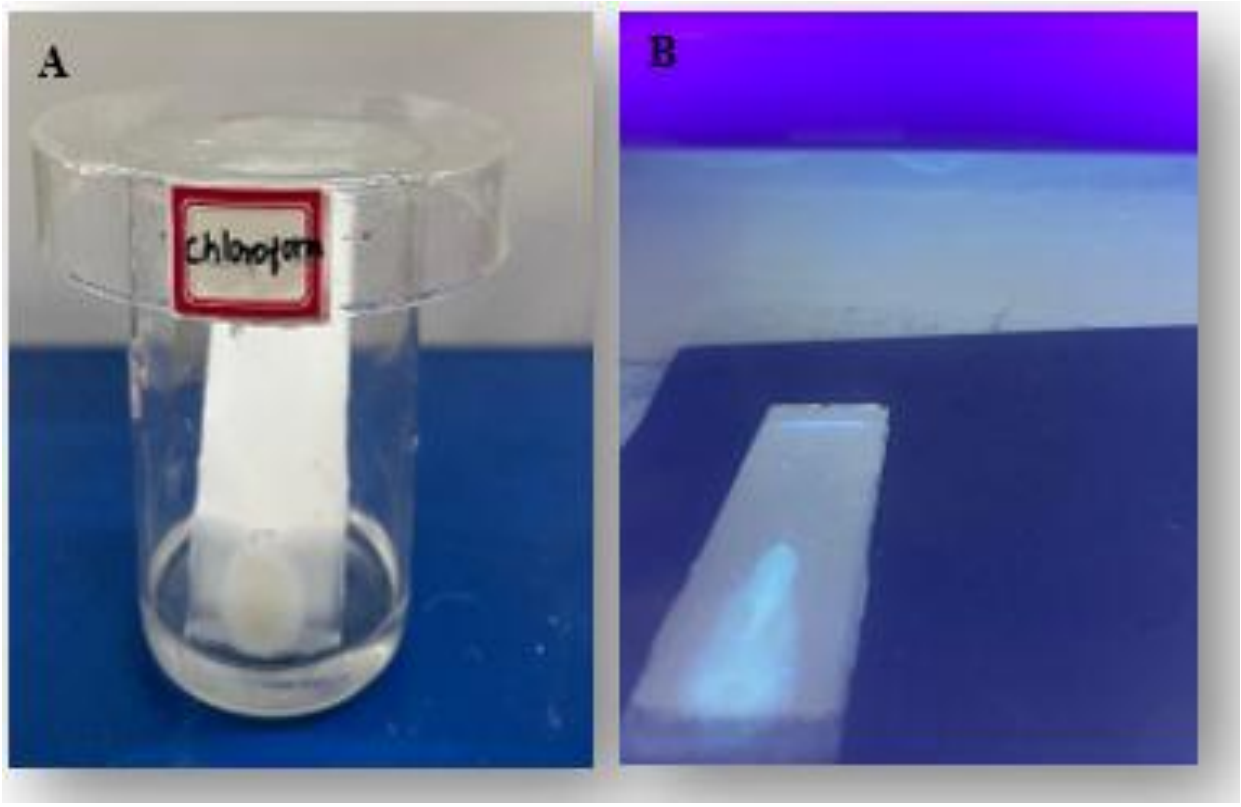

Fig 10. Thin Layer Chromatographic analysis of Chloroform: A. Loaded sample in mobile phase, B. TLC plate under UV light

Table.03: Thin Layer Chromatographic analysis of Oyster Mushroom extracts with their Rf values in different solvent systems.

SOLVENT SYSTEM	EXTRACT	NUMBER OF BANDS	R_F VALUE
BUTANOL: ETHANOL: ACETONE	Standard	01	0.84
BUTANOL: ETHANOL: ACETONE	Ethyl acetate	01	0.42
BUTANOL: ETHANOL: ACETONE	Chloroform	01	0.92

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum was used to identify the functional group of the active components of mushroom strains based on peak value in the region of inferred radiation. FTIR peaks categorized changes in nutritional profile of crude extract, ethyl acetate and chloroform extracts of oyster mushroom. Extracts showed variation in the spectral area of the key biochemical compounds, i.e. proteins, fatty acids and carbohydrates. The FTIR spectra demonstrated comprehensive nutritional profile of oyster mushroom. There are many high peaks were observed, in that one important broad band in standard were located between 3500-3000cm^{-1}is the result of the hydroxyl group (O-H) around3322cm^{-1}, signalling the presence of polysaccharide chains intra and intermolecular interaction **(Sowmya *et al.*, 2023)**. In the standard sample and it is similar in ethyl acetate and chloroform extract, it means that ethyl acetate and chloroform extract also having the hydroxyl (O-H) bond, overlapping with N-H amide stretching vibration around 3270-3100cm^{-1}. The peak appearing around 2948-2835cm^{-1} in standard and at 29855cm-1 in ethyl acetate have been assigned to the C-H stretching of CH_2 and CH_3 group are the characteristics of lipids as showed in the figure 11, but not in chloroform extract due to the volatile nature of the solvent. According to Focher, the standard sample shows lower resolution of amide I around 1651cm^{-1}-1553cm^{-1} is possibly due to the methanol and high content of beta-glucan **(Killedar *et al.*, 2025)**. However, in ethyl acetate and chloroform the amide I band shows high resolution around 1735-1738 cm^{-1}. The relative intensity between amide I band at 1637-1738 cm^{-1}. Beta-glucan band at 1043cm^{-1}is higher in ethyl acetate spectra than in standard, similar to the bands at 1651cm^{-1} and 1010cm^{-1}. Probably related to high degree of acetylation (70.79%) in ethyl acetateand it proves that chloroform extract has a lower amount of chiin than the amount of beta-glucan**(Pestov *et al.*, 2009)**, despite that quantitative estimates were difficult to determine using infrared analysis. In standard and chloroform extract spectra the band at 1449-1453cm^{-1} indicates the presence of carboxy or ester (C=O). This bands appears together with two sharp bands near 2985cm^{-1} and 2835cm^{-1}(CH_2in long alkyls) all these features are charaacteristics of lipids.

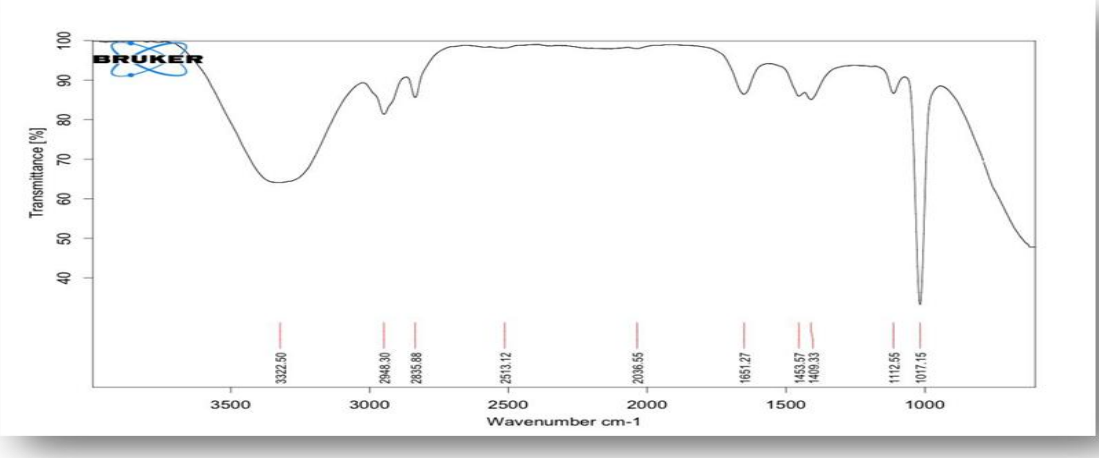

Fig 11. FTIR analysis of Standard

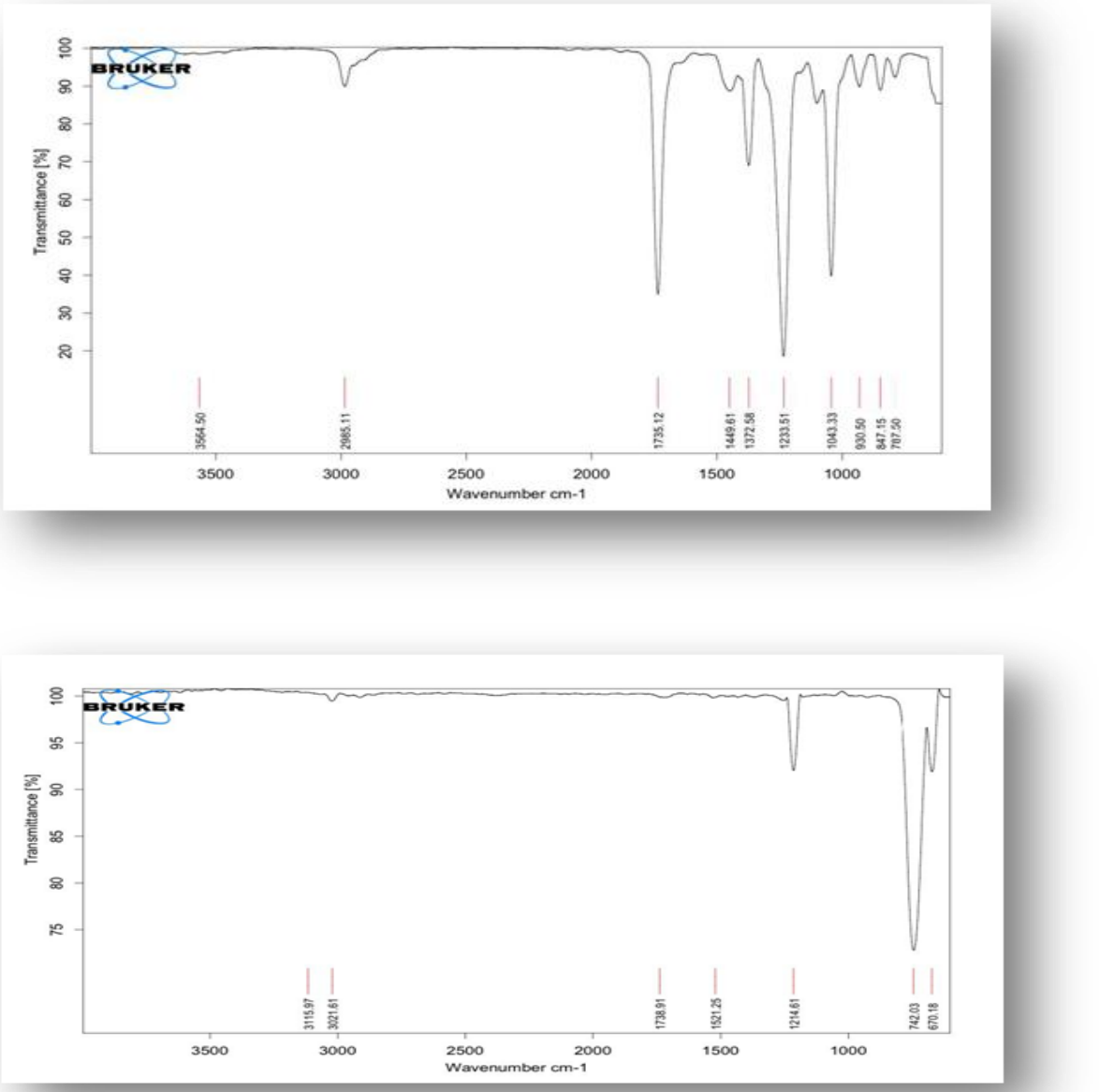

Fig 12. FTIR analysis of Ethyl acetate and Chloroform

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) of the microstructure of dried oyster mushrooms can be compared visually by referring to Figs.14, 15 and 16. From the SEM results obtained, the surface of standard, ethyl acetate and chloroform extract of dried mushroom exhibited high porosity. The surface images of ethyl acetate and chloroform extract is more rigid than standard. Ethyl acetate extract surface showed bigger pore size due to the observation that hot water boiling circulation has caused a rapid moisture transfer which has leads to swelling of the cells and larger channels being formed inside the sample. This explains, the size of the pore of ethyl acetate dried mushroom is bigger compare to standard and chloroform. There is a less pore that can be seen in standard as well as in chloroform and it has smoother surface. In chloroform sample due to the versatile nature of mobile solvent, chloroform sample appears shiny and smooth comparatively ethyl acetate.

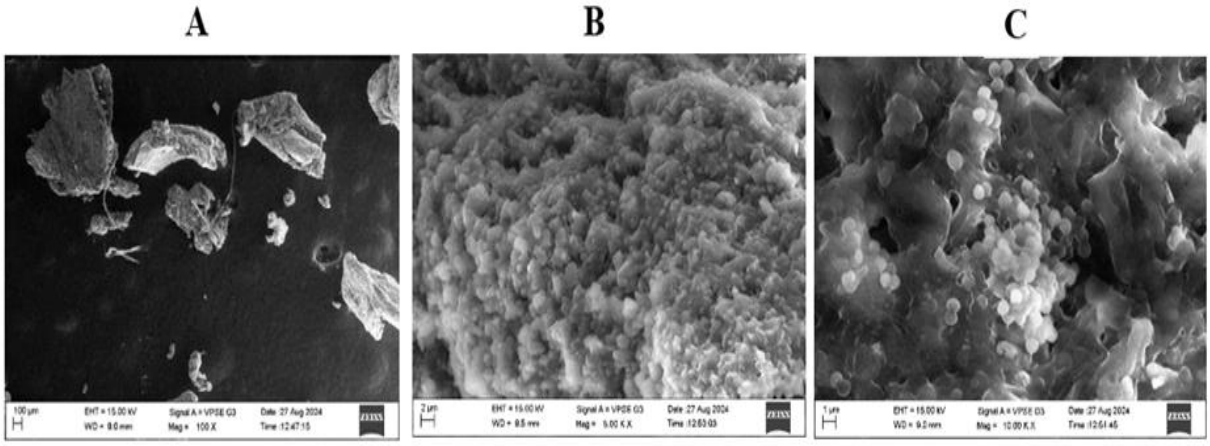

Fig 14. SEM images of the surface morphological structure of a standard sample. Voltage 15.00kVMagnification A:100X, B: 5.00X, C: 10.00X.

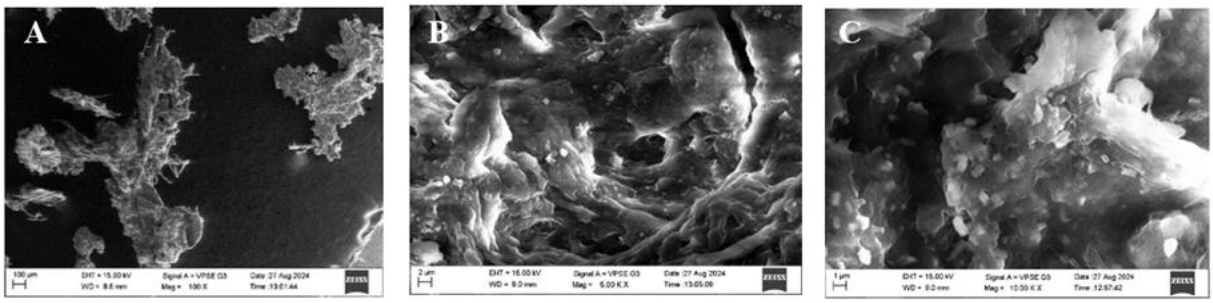

Fig 15. SEM images of the surface morphological structure of an Ethyl acetate sample. Voltage 15.00kV Magnification A:100X, B:5.00X, C: 10.00X

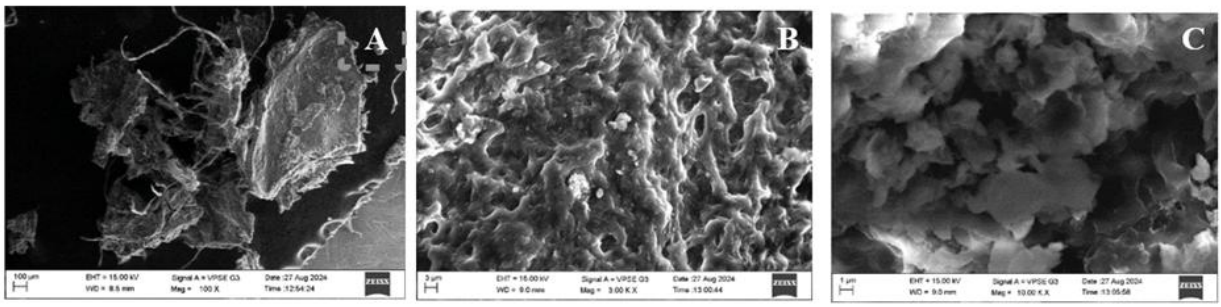

Fig 16. SEM images of the surface morphological structure of a Chloroform sample. Voltage 15.00kV, Magnification A: 100X, B: 5.00X, C: 10.00X.

IV. DISCUSSION

According to Venkatesa., (2019) Mushroom belongs to the group of organisms known as macrofungi under the phylum Ascomycotina and Basidiomycotina. Mushroom is the fleshy and spore - bearing organ of the fungi that called as fruiting body. Mushrooms are seasonal fungi, which occupy diverse niches in forest and territory ecosystem. They mostly occur during the rainy season, particularly in forests, where the dense canopy shade from trees provides a moist atmosphere and decomposing organic material such as leaf litter, and favors the germination and growth of mushrooms. Gobert.,(2015) Making a spore print is a crucial step in correctly identifying mushrooms. The colour of the spore print is one of the most reliable distinguishing

features for differentiating mushroom species, especially those that may resemble each other in shape and cap colour. In addition to its usefulness for identification, spore fingerprinting is also valuable for the study of biodiversity and scientific research, helping to understand the distribution and ecology of different species of fungi. According to Okafor., (2017). I have performed DPPH being a stable free radical accepts electrons or hydrogen radicals to become stable diamagnetic molecules. The reaction of ethyl acetate and chloroform extract with purple coloured DPPH radical, is convert the radical α, α diphenyl-β picrylhydrazine due to the extract antioxidant property. The presented SEM images proved to be good evidence of structural changes resulting from standard, ethyl acetate and chloroform extract Piskov (2022). The surface images of ethyl acetate and chloroform extract is more rigid than the standard while there is a less pore can be seen in standard as well as chloroform and it has smoother surface Acay (2019).

V. CONCLUSION

Mushrooms are gaining popularity due to their high nutrient content, bioactive compounds, and health benefits. They have antihypercholesterolaemic, antiviral, anticancer, and antihypertensive activities. The food and pharmaceutical industries are using mushrooms or their bioactive compounds to develop functional foods. A study on wild edible mushrooms, *Pleurotus ostreatus*, found that its polysaccharides could be used as a natural antioxidant drug resource, further research is necessary to explore the specific mechanisms by which these mushrooms exert their antioxidant effects and to assess their long-term benefits in human health. As awareness of the importance of antioxidants grows, wild mushrooms may play a crucial role in dietary strategies aimed at reducing oxidative stress and improving overall health.

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