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Research Article

The Ecological and Medicinal Significance of *Agaricus bisporus:* Phytochemical Insights into Its Role in Nutrient Cycling and Health Promotion

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ABSTRACT

Mushrooms, classified as fleshy, spore-bearing fungi, thrive in diverse environments and play crucial roles in nutrient cycling and organic matter decomposition. Although often regarded as vegetables, they are biologically distinct from plants and can be both edible and toxic. Noting their use in ancient cultures and their growing recognition in contemporary research for their bioactive compounds that exhibit anticancer and immunomodulatory properties. The collection and extraction of mushroom samples from various locations in Davangere, followed by a systematic approach to phytochemical analysis. The extraction process involved using ethyl acetate to isolate bioactive compounds from the mushroom powder. Various qualitative tests were conducted to identify the presence of key phytochemicals such as alkaloids, glycosides, terpenoids, and flavonoids, a rich profile of bioactive constituents with potential health benefits.

Keywords*: Phytochemical Analysis, Bioactive Compounds, Nutraceuticals, Ecological Role, Immunomodulatory properties.*

INTRODUCTION

A mushroom is a fleshy, spore-bearing fruiting body of a fungus, produced on soil above the ground, and comes in a wide range of varieties (Priyadarshni et al., 2022). Mushrooms can be found growing in open areas, rubber and oil palm growing areas, primary and secondary forests, lowlands, and highlands (Shahriari et al., 2019). Even though mushrooms are categorized as vegetables in the food industry, they are not actually plants. Nevertheless, they offer a number of vital nutrients, some of which are edible and some of which are extremely toxic and hallucinogenic.

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They play a vital role in forest ecosystems as saprobes, pathogens, and symbionts. Mushrooms have high nutritional value with low calorie content and the best quality vitamins and proteins (Aamlid et al., 2022). It can be utilized as an alternative source of medicine for its curative properties. They are a potent source of nutraceuticals and various bioactive molecules, which bear anticancer and immunomodulatory properties. Mushrooms are achlorophyllous (lack chlorophyll), and hence cannot prepare food; they get their food from decomposing dead, decaying organic matter. So, mushrooms are also called saprophytic fungi, which is a type of heterotrophic mode of nutrition. Mushrooms have been a part of the human diet in many regions of the world for centuries due to organoleptic characteristics as well as nutritional characteristic values (Waqas et al., 2019). Traditionally, mushrooms were used as a food source or a remedy for their known healing properties. Ancient Egyptians valued mushrooms, which they referred to as the "food of God," and only pharaohs were permitted to consume mushrooms (Ashok et al., 2017). Studying and investigating the biological activities of mushrooms has gained popularity recently (Fonseca et al., 2024). The number of mushroom species was estimated to be around 1.5 million. However, it is believed that the actual number is around 2.2 to 3.8 million worldwide. Out of this number, only about 14000 species have been identified, 1400 of which are macro fungi, 1069 are considered, and 700 species were found to have therapeutic properties (Buruleanu et al., 2018).

MATERIALS AND METHODS

Collection and Extraction of Samples:

The fresh mushrooms were collected from the campus in Davangere. Further samples were transferred to sterile polythene zip lock bags, and the samples were carried with aseptic conduction to the Department of Microbiology laboratory of Davangere University to perform further experiments (Ketemepi et al., 2024). The collected mushrooms were first washed with water thoroughly to be free from mud, ferns, and other extraneous material, then the mushrooms were destalked, washed, and sun-dried. Then dried on blotting paper to remove moisture, the mushroom was dried at room temperature for 48 hours (Sirohi et al., 2024). Then the dried mushroom was cut into small pieces about 5-10 mm in length, and it was subjected to grinding. After the grinding, fine powder was obtained, and it was stored in an air tight jar (Courtney et al., 2012). 5 gm of mushroom powder was weighed and soaked in 50 ml of ethyl acetate and incubated for 24-36 hours at room temperature. Extracts were filtered with Whatman filter no. 1, and the light brownish extract was used for phytochemical analysis (Ikon et al., 2018).

Phytochemical Analysis of *Agaricus bisporus* extract

The test extract is subjected to 8 qualitative phytochemical tests that are carried out to check the presence of bioactive compounds according to Killedar et al. 2024**.**

Test for Alkaloids

Mayer's test: To 2 ml of test solutions, a few drops of Mayer's reagent (potassium mercuric iodide solution) were added, and the solutions were observed for turbidity or precipitation. The appearance of cream-colored precipitate signifies the presence of an alkaloid.

Test for Cardiac Glycosides (Keller-Killiani test): 2 ml of test solutions, 1 ml of glacial acetic acid, and 1-2 drops of ferric chloride solution were added. Then, 0.5 ml of concentrated sulfuric acid was slowly added along the sides of the test tube, avoiding shaking of the test tube. A reddish-brown ring at the interface of two liquids indicates the presence of deoxysugar characteristic of cardenolides.

Test for Tannins and Phenolic Compounds (Lead Test): To 2 ml of the test solutions, 1-3 drops of ferric chloride were added, and the mixture was observed for blue or green color.

Test for Saponins (Foam Test): The test solutions are taken in a test tube and shaken vigorously until a stable, persistent froth is obtained. The persistence of froth for 10

minutes signifies the presence of saponins (Udu-Ibiam et al., 2014).

Test for Terpenoids and Phytosterols (Salkowski test): To 0.5 ml of test solution, add an equal quantity of chloroform and then add sulfuric acid along the sides of the test tube, avoiding shaking. The appearance of color in the chloroform layer signifies the presence of phytosterols.

Test for Flavonoids:

A 10% lead acetate solution is added to 1 millilitre of the test solutions in the lead acetate test (1.2.6.1). The appearance of yellow-colored precipitate signifies the presence of flavonoids.

$FeCl_3$ test: To 2 ml of the test solutions, add ferric chloride solution drop by drop. The formation of a greenish-black color indicates the presence of flavonoids.

Test for Proteins (Biuret test): Add 2 ml of Biuret reagent to 2 ml of the test solutions. Shake well and warm it in a water bath. The appearance of red or violet color indicates the presence of proteins.

Test for fixed oils and fatty acids (spot test): A drop of extract was added to the filter paper and observed. Oil staining on the filter paper indicates the presence of fixed oil and fats (Ramalingappa et al., 2025).

Analysis of Total Carbohydrate Content by Using Phenol-Sulphuric Assay

10 ml of Dextrose solution was prepared and incubated in a water bath at 100°C for 30 mins (Killedar & Ramalingappa, 2024). The solution was cooled down, and sodium carbonate was added until the effervescence stopped. Dextrose solution was then filtered using Whatman No.1 filter paper, and the volume was made up to 10 ml using distilled water. 1 ml of the standard series was prepared with a concentration 200-1000µg/ml. Distilled water was added to each test tube by makeup to 1 ml, then 1ml of 5%phenol solution was added to each standard series and 1 ml of mushroom ethyl acetate extract. The mixtures were incubated for 10 minutes at room temperature and then incubated again in a water bath at 27°C for 20 minutes until a reddish green color appeared. Absorbance was measured at 400 nm using the UV-Vis Spectrophotometer. A standard curve was plotted using the absorbance of the standard series, and the total carbohydrate content of mushroom samples was derived (Bose et al., 2019).

Thin Layer Chromatography

The mushroom ethyl acetate extract was examined to check the bioactive compound content by thin-layer chromatography (TLC). The TLC slide was developed by using benzene (6 ml), ethanol (2 ml), and acetone (2 ml) as a solvent system. The chromatographic chamber with solvent was kept for 15 minutes for equilibration. The sample was spotted on a silica gel slide with the help of a capillary tube. The slides were placed in a solvent system and allowed to run; the slides were observed under UV light. Retention factor (R_f) value was calculated according to the following equation from the chromatogram (Hoosseini et al., 2024)

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

High-performance liquid chromatography (HPLC):

200 μl of 70% methanol was filtered through a 0.4 μm filter to remove all suspended particles. After filtration, it was used for HPLC. Polyphenols present in the samples were detected. The chromatographic system consisted of a C-18, 5 μm silica column (250×4.6 mm) at 40°C. The injection system used was a 20μl sample loop. 20μl of crude extract and 10μl of ethyl acetate extract, respectively, were loaded. Detection was done by a UV-visible spectrophotometer set at a wavelength of 280 nm. Gradient HPLC carried out the separation of antioxidant components with a mobile phase composition ranging from 2 to 80% Acetonitrile with 2% acetic acid in water. Gradient elution was at a rate of 0.5ml/min with a gradient program (0- 40 min, 2- 40%, B; 40-50 min, 40% B; 50-60 min, 40-50% B) with 2% Acetic acid in water as solvent A and Acetonitrile as Solvent B. The

column was equilibrated between injections for 10 min with the initial mobile phase (Killedar et al., 2024).

Scanning electron microscopy (SEM):

To conduct an SEM analysis of an ethyl acetate mushroom liquid extract, the liquid must first be converted into a solid or semi-solid form. This is typically done by evaporating the ethyl acetate solvent through methods such as rotary evaporation or freeze-drying. The resulting solid residue, or precipitate, is collected and may undergo additional preparation, such as fixation with glutaraldehyde for biological components, though this step is optional. After dehydration, if necessary, the dried sample is mounted on an aluminium stub using conductive adhesive tape. The mounted sample is then sputter-coated with a thin layer of a conductive material, like gold or platinum, to prevent charging during SEM imaging. Once prepared, the sample is placed into the SEM chamber, and imaging parameters such as accelerating voltage and working distance are adjusted based on the sample's characteristics. Images are captured at different magnifications to study the surface morphology, crystalline structures, or other microscopic features present in the mushroom extract (Anbazhagi et al., 2025).

RESULTS AND DISCUSSION

The Sample Collection and Extraction

Fresh mushrooms were collected from the Davangere district (Davangere University campus). Then the samples were transferred to sterile polythene zip-lock bags. (Pipriya et al., 2019) Then, the samples were carried to the Department of Microbiology laboratory of Davangere University to carry out further experiments. They were cleaned and washed with running tap water, washed and cut into small pieces, dried for 48 hours, and then ground into powder. Dried powder was then used for the experimental purpose, as shown in (Killedar et al., 2024).

Fig 1: Collection of *Agaricus bisporus,* Davangere University campus, Shivagangotri,
A- *Agaricus bisporus* in the lab conditions; B- Cleaning *Agaricus bisporus* with running tap water;
C-D. Drying the *Agaricus bisporus* with tissue paper to remove unwanted water;
E- Grinding the powder of the air-dried *Agaricus bisporus* in the zip bag

Solvent extraction by the ethyl acetate method

5 gm of mushroom powder was weighed and soaked in 50 ml of ethyl acetate solvent and incubated for 36 hours at room temperature. After the incubation, the extract was filtered with a Whatman filter no. 1, and the extract was stored in an air-tight container as shown in Fig. 2 and used in the phytochemical analysis of *Agaricus bisporus*.

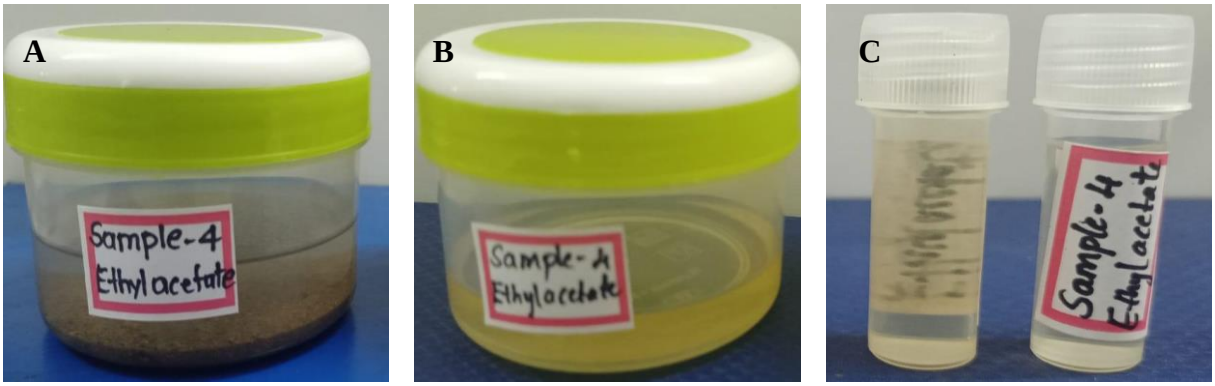
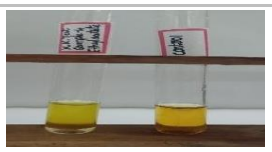
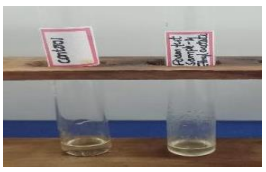
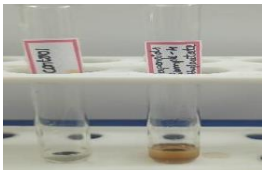
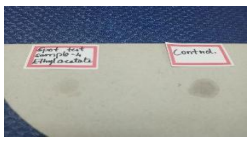


Fig 2: The Extraction step of the ground powder of the air-dried *Agaricus bisporus*;
A- Powder of *Agaricus bisporus* into ethyl acetate in the ratio of 5 g to 50 ml;
B- After the 24-36 hours of incubation, the yellowish solvent extract;
C- The yellowish solvent of *Agaricus bisporus* is subjected to phytochemical analysis for the bioactive compounds

Phytochemical Analysis of *Agaricus bisporus* extract.

The phytochemical analysis of *Agaricus bisporus* confirmed the presence of phytoconstituents such as alkaloids, glycosides, terpenoids, flavonoids, and fatty acids through various chemical tests. Notably, tannins, flavonoids, and silently proteins were present, as indicated by the test analysis (Kutluer et al., 2024). Phytochemical analysis was conducted to determine antioxidant or bioactive compounds, as shown in Fig. 2. The results were tabulated in Tab. 1. Killedar et al. conducted the phytochemical screening, which revealed the presence of various bioactive compounds, including alkaloids, glycosides, tannins, terpenoids, and flavonoids. Interestingly, the lack of fatty acids and fixed oils points to a particular composition of bioactive ingredients. Phytochemical analysis and antimicrobial activity studies of mushrooms have gained significant attention due to their medicinal potential, bioactive compounds, numerous secondary metabolites, including polysaccharides, phenolic compounds, terpenoids, alkaloids, and steroids, are found in mushrooms and contribute to their biological activities. (Kothiyal et al., 2022) Phytochemical analysis involves the identification of bioactive compounds present in mushrooms. These bioactive substances have antibacterial, anti-inflammatory, and antioxidant qualities, among other therapeutic effects. According to Courtney et al. (2012), the bioactive compounds in mushroom tissue are extracted using a variety of solvents, such as methanol, ethyl acetate, chloroform, acetone, or water. The choice of solvent can affect the efficiency of the extraction, as different compounds have varying solubilities.

Table 1: Phytochemical analysis of *Agaricus bisporus*

Bioactive compound	Tests	Ethyl acetate Extract	Figure of Results
Alkaloids	Mayer's test	Positive	
Cardiac Glycosides	Keller-Killiani test	Positive	
Tannins	Lead test	Positive	
Saponins	Foam test	Negative	
Terpenoids	Salkowski test	Positive	
Flavonoids	Lead acetate test	Positive	
	Ferric chloride test	Negative	
Proteins	Biuret test	Positive	
Fatty acids	Spot test	Positive	

1.4. Analysis of Total Carbohydrate Content by Using the Phenol-Sulphuric Assay

10 ml of Dextrose solution was prepared and incubated in a water bath at 100°C for 30 mins. The solution was cooled down, and sodium carbonate was added until the effervescence stopped. Dextrose solution was then filtered using Whatman No.1 filter paper, and the volume was made up to10 ml using distilled water. 1ml of the standard series was prepared with a concentration of 200-1000 µg/ml. Add distilled water to each test tube by makeup to 1 ml, then 1 ml of 5%phenol solution was added to each standard series and 1 ml of mushroom extracts (ethyl alcohol and chloroform extracts). The mixtures were incubated for 10 minutes at room temperature, and then incubated again in a water bath at 27°C for 20 minutes until a reddish green color appeared. Absorbance was measured at 400 nm using the UV-Vis Spectrophotometer. A standard curve was plotted using the absorbance of the standard series, and the total carbohydrate content of mushroom samples was derived (Tamboli et al., 2020).

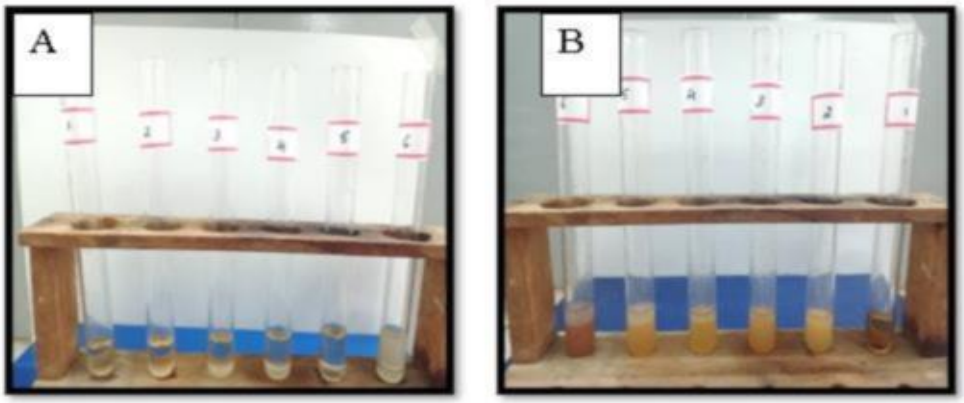


Fig.3. Image A: Showing the test tube before incubation at room temperature, and Image B: Test tube after the incubation, and the absorbance was taken at 400 nm to check the total carbohydrate contents of the ethyl acetate extract of *Agaricus bisporus*

Table 2: Quantitative evaluation of the total amount of carbohydrates in the Agaricus bisporus mushroom's ethyl acetate extract using the phenol sulphuric assay.

SI.No	Dextrose solution (in ml)	Distilled water (in ml)	5% phenol solution (in ml)	Mushroom extract (in ml)			Concentration	OD at 440 nm
1	0	1	1	1	Incubate for 10 mins at room Temperature	Incubate in a water bath at 27 °C for 20 mins	0	0
2	0.2	0.8	1	1			200	1.32
3	0.4	0.6	1	1			400	1.40
4	0.6	0.4	1	1			600	1.55
5	0.8	0.2	1	1			800	1.76
6	1	0	1	1			1000	1.77

Thin Layer Chromatography:

The TLC slide was developed, and after 15 min of equilibration, the sample was spotted on a silica gel slide, and the slides were allowed to run and observed under UV light. The ethyl acetate extract and standard extract of mushrooms show the presence of bioactive compound content by showing bands under UV light, as shown in fig 13. The R_f value of the Ethyl acetate extract is 0.85; the R_f value of the standard extract is 0.86. (Fig.03, Table-03).

Fig.4. Depict 1 and 2: Ethyl acetate extract under the UV-Visible light to observer the bands, and for comparison the crude extract of the *Agaricus bisporus* depicted in Fig.5 and 6 showing the similar bands in both the extracts

Table 3: Thin Layer Chromatographic analysis of *Agaricus bisporus* extracts with their Rf values in the solvent system.

Solvent System	Samples	Retention factor value
Benzene: Ethanol: Acetone 6:2:2	Ethyl acetate extract	0.85
	Standard extract	0.86

High Performance Liquid Chromatography (HPLC):

A 0.4 μm filter was used to filter 200 μl of 70% methanol in order to eliminate any suspended particles. Following filtration, HPLC was employed. It was found that the samples contained polyphenols. A C-18, 5 μm silica column (250×4.6 mm) operating at 40°C comprised the chromatographic apparatus. A 20μl sample loop was the injection system that was utilized. 10μl each of standard extract and ethyl acetate, as well as 20 μl of crude extract, were loaded. A UV-visible spectrophotometer with a wavelength of 280 nm was used for detection. Gradient HPLC was used to separate the antioxidant and bioactive components, with a mobile phase composition of 2-80% acetonitrile (A) and 2% acetic acid in water (B). Using a gradient program (0- 40 min, 2- 40%, B; 40-50), gradient elution was carried out at a rate of 0.5 ml/min (Fig.5, Fig.6).

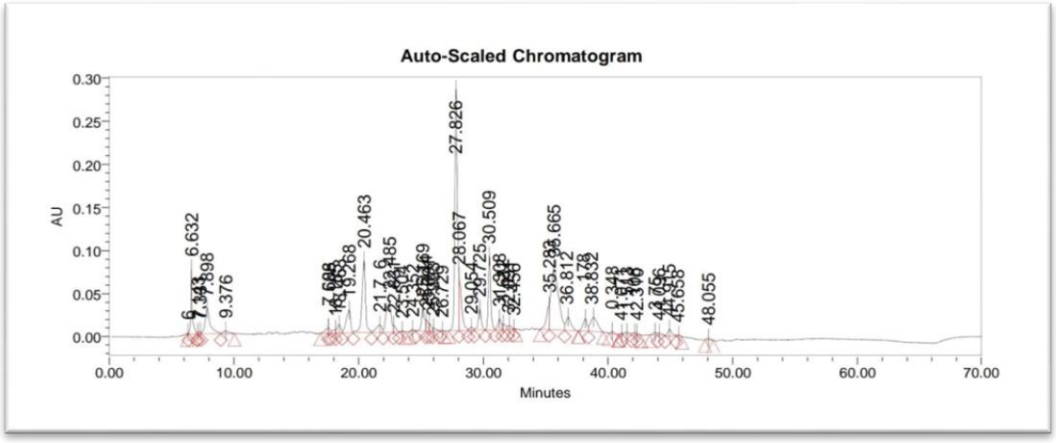


Fig.5. HPLC analysis of the standard extract of *Agaricus bisporus* shows the peak in 0.30AU and showing the different bioactive components based on peak value

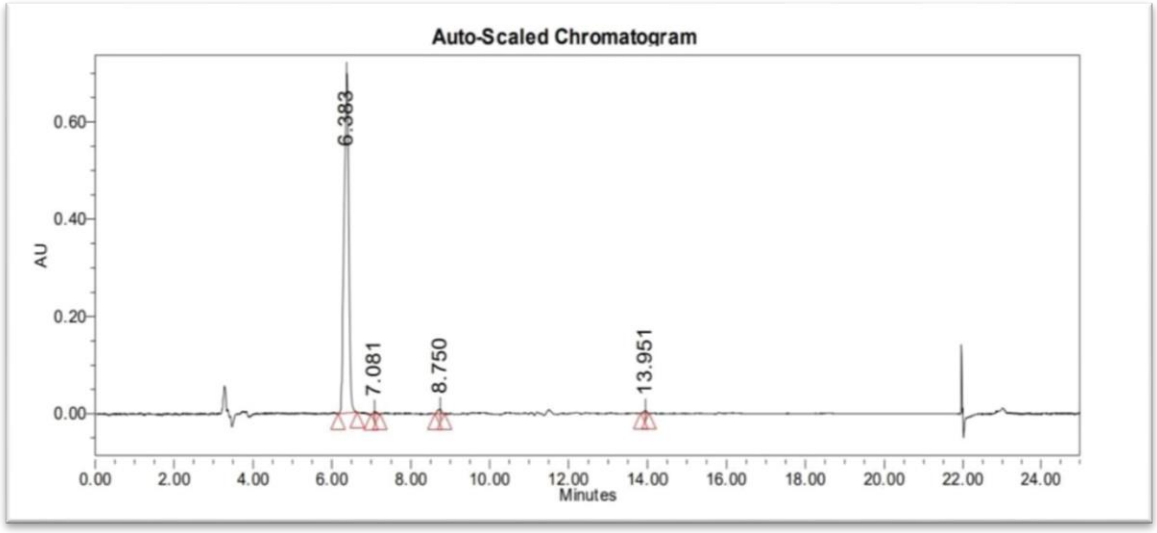


Fig.6. HPLC analysis of the ethyl acetate extract of *Agaricus bisporus* shows the peak in 0.30AU and showing the alkaloids, glycosides, terpenoids, and flavonoids

Scanning Electron Microscopy:

Scanning Electron microscopy (SEM) of the microstructure of dried button mushrooms can be compared visually by referring to Fig. 7. From the SEM results obtained, the surface of *Agaricus bisporus* standard, ethyl acetate mushroom exhibited high porosity. The surface images of ethyl acetate and the standard are more rigid. The ethyl acetate extract surface showed a bigger pore size due to the observation that dried circulation gas caused a rapid move in transfer, which has led to swelling of the cells and larger channels being formed inside the sample. This explains that the size of the pore of the ethyl acetate-dried mushroom is bigger compared to the standard. There is a smaller pore that can be seen in the standard, and it has a smoother surface.

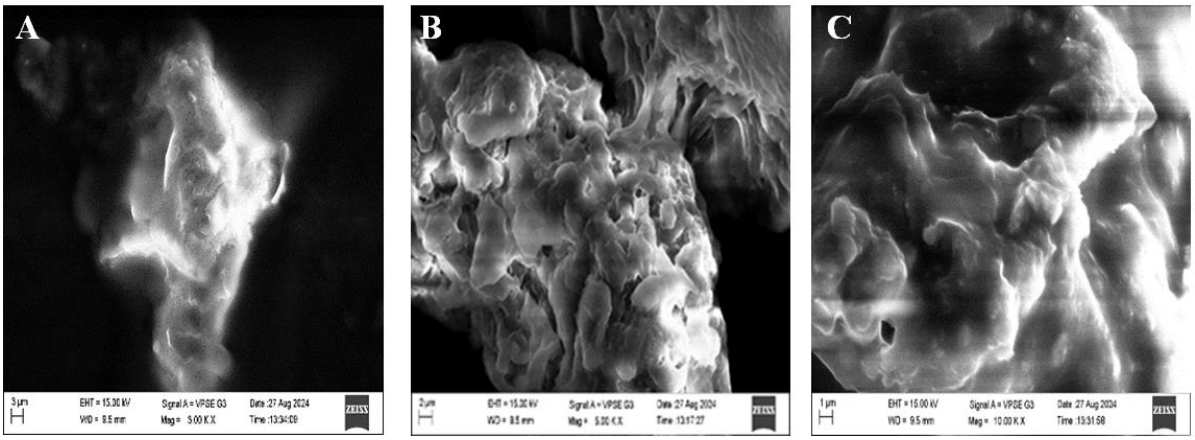


Fig.7. Depicted the surface morphology of the Ethyl acetate extract of *Agaricus bisporus* was observed using a Scanning Electron Microscope at an excitation voltage of 15 kV at 10 KX, Fig A. Magnification under 3.00 KX, Fig B. Magnification under 5.00 KX and Fig C. Magnification under 10.00 KX

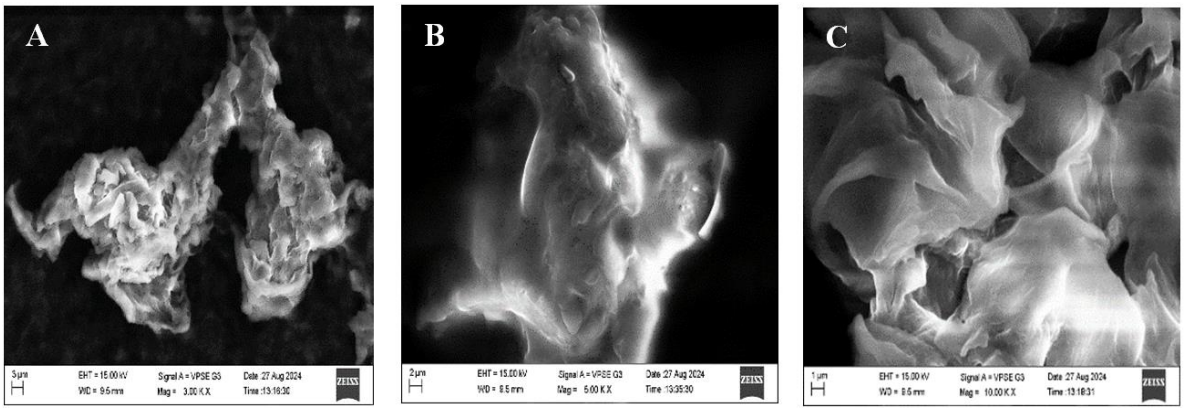


Fig.8. Depicted the surface morphology of the standard of *Agaricus bisporus* was observed using a Scanning Electron Microscope at an excitation voltage of 15 kV at 10 KX, Fig A. Magnification under 3.00 KX, Fig B. Magnification under 5.00 KX and Fig C. Magnification under 10.00 KX.

CONCLUSION

Mushrooms, diverse in shape, size, color, and texture, play a crucial ecological role by decomposing organic matter and recycling nutrients. They contribute to soil health and nutrient cycling. A phytochemical analysis of mushroom extracts identifies bioactive compounds, including polysaccharides, phenolic compounds, triterpenoids, sterols, flavonoids, terpenes, and alkaloids. Mushroom compounds, including polysaccharides and peptides, Research into these compounds could lead to new antibiotics and treatments for drug-resistant infections, enhancing their potential applications in healthcare, food production, and environmental management. Phenolic compounds in mushrooms have strong antioxidant and antimicrobial properties, potentially reducing chronic disease risk and promoting overall health. Flavonoids, another significant group of phytochemicals, have anti-inflammatory and antimicrobial effects, disrupting microbial cell membranes and inhibiting enzyme activities, enhancing the therapeutic potential of mushrooms against various microbial pathogens. Mushrooms contain alkaloids and terpenoids, which have antimicrobial properties. Alkaloids interfere with microbial metabolic pathways, while terpenoids affect cell membranes and processes. It provides a comprehensive overview of the significance of mushrooms, shedding light on their historical importance, nutritional value, and medicinal properties. It emphasizes the role of mushrooms in the forest ecosystem and their traditional use as food and medicine.

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Conflict of Interest: The author declares that there are no conflicts of interest.

Author Contribution: Dr. Ramalingappa B., Senior Professor, supervised the lab and designed the methodology.

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