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Chemical Examination of Phytoconstituents in Edible White button mushroom [*Agaricus bisporus*]: A Potential medicinal Plant

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Abstract

The present study includes isolation, chemical examination nutritional value and medicinal importance of white button mushroom. Significant secondary metabolites were found in *Agaricus bisporus* after a qualitative phytochemical screening in this investigation. Flavonoids, phenols, saponins, glycosides, and tannins are examples of phytochemical substances that are thought to be important secondary metabolites in mushrooms. Alkaloids, carbohydrates, glycosides, proteins, phenols, flavonoids, saponins, terpenoids, cardiac glycosides, tannins, and steroids were all detected in the extracts during the initial screening process. According to HPLC, the main components in the methanol extract of *A. bisporus*were gallic acid and ergothioneine, respectively. These active metabolites are well known for their ability to treat a variety of human conditions, including menstrual disorders, chronic eczema, diarrhoea, dysentery, spasmodic, and diuretic-choleretic. Depending on the molecular formula, retention time-molecular weight, and peak area, our HPLC data indicates the existence of various bioactive substances. Its antibacterial, antidiabetic, and antioxidant qualities may be attributed to the primary phytochemical components found in *A. bisporus,* which have been identified in the current work.The initial results demand more thorough research, including in vitro tests. Thus, it is possible that the mushroom employed in this study will find application in the pharmaceutical business for a variety of medical purposes.

Key words : *Agaricus bisporus*, HPLC analysis, Antioxidant, Phytochemicals, Phenolic compounds.

Introduction

Agaricus bisporus commonly known as the edible white button mushroom or cultivated mushroom. It is basidomycete , mushroom native to grass land in Eurasia and north America ,it is cultivated in more than seventy countries and is one of the most commonly and widely consumed mushroom in the world. *Agaricus bisporus*, also known as the button mushroom, is one of India's most widely farmed edible mushrooms. They grow naturally in grasslands and pastures and are nutrient-rich. In the present study, qualitative phytochemical analyses were performed on *Agaricus Bisporus*.

Plants are widely recognised for their value. The plant kingdom is a goldmine of possible medications, and the value of medicinal plants has gained attention in recent years. Plant-based drugs are widely available, less expensive, safer, and more efficient, with less adverse effects. Plants that have been selected for medical use over thousands of years are the most obvious choice when investigating the current quest for therapeutically effective novel medications such as anticancer pharmaceuticals[1], antibacterial drugs[2], and antihepatotoxic chemicals.

According to the World Health Organisation (WHO), medicinal plants are the most excellent source for obtaining a variety of medications. Approximately 80% of people in developed nations utilise traditional medicines, which contain substances derived from medicinal plants. To learn more about these plants' characteristics, safety, and effectiveness, further research is necessary[3]. Medicinal plants contain chemical components that have a specific physiological function on the human body, such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids[4,5].

Via primary or more accurately secondary metabolism, these chemicals are produced in organisms. Secondary metabolites are chemically & taxonomically exceptionally differed substances with unexplained functions. They are widely employed in human therapy, veterinary medicine, agriculture, scientific research, and a variety of other applications[6]. In vitro, a wide range of phytochemicals from various chemical families have been found to suppress the growth of all types of bacteria[7]. Plant materials have been used in phytomedicines since ancient times. This can be obtained via barks, leaves, flowers, roots, fruits, and seeds[8]. Knowing the chemical elements of plants is desirable since it will be useful for the production of complicated chemical substances[9,10,11].

In the past few decades, people have classified mushrooms based on their potential for therapeutic use. Today, contemporary scientific research is confirming traditional remedies and investigating their potential for producing new pharmaceutical treatments. This has sparked an increased interest in mycology including the study of therapeutic mushrooms. Researchers have discovered a number of bioactive compounds that have showed promise in treating a variety of illnesses, including cancer, immunological disorders, and neurological diseases. *Agaricus bisporus*, also known as the button mushroom, is one of India's most widely farmed edible mushrooms. They grow naturally in grasslands and pastures and are nutrient-rich[12]. In the present study, qualitative phytochemical analyses were performed on *Agaricus Bisporus*.

Preparation extracts of white button mushroom:

5gm of dried finely powdered plant material was taken in a beaker and 200ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30º-40ºC for 20 minutes. Then the water extract was filtered through filter paper and the filtrate was used for

the phytochemical analysis. The water extract was kept in refrigerator for further use.

Chemical examination of Agaricus bisporous:

The extract was analysed for qualitative phytochemical screening as per the standard protocol mentioned below :

Test for Terpenoids : 0.5 ml crude extract added to 2 ml chloroform + 3 ml sulphuric acid ,formation of reddish-brown colour indicates presence of Terpenoids.

Test for Phenol : To 10mg ofcrude extract added 1 mL of leadacetate. Formation of precipitate indicates presence of phenolic compounds.

Test for Tannins : 200mg plant extract is boiled with 10 ml distilled water, then 0.1% of ferric chloride is added and mixed. A bluegreen or black colour indicates presence of tannins.

Test for Saponins :

To 1 mL of extract added 5 mL of distilled water, shaken well and formation of foam indicates presence of saponins.

Test for Flavonoids : Few drops of 10% lead acetate were added to10 mg of plant extract, yellowcolour precipitate indicates presence of Flavonoids.Few drops of 10% NaoH is added to 5mg plant extract, watery yellow colour indicates the presence of Flavonoids.

Test for Steroids : 1 mL of extract mixed with 10 mL of chloroform and 10ml H_2SO_4. Upper layer turns red and H_2SO_4 layer showed yellow colour indicates presence of steroids.

Test for Alkaloids was done by Wagner reagent test- Preparation of Wagner reagent :- Potassium iodide (2gms) + iodine (1.24gm)+ DD Water(5ml)Solution is diluted to 100 ml**.** Few drops of wagers reagent + 10 mg of plant extract**.** Reddish brown precipitate indicate the presence of Alkaloids.

Preparation of Sample :

For analysis of *Agaricus bisporus*powder sample, extraction was done using soaking method and in that 1gm sample powder was mixed with 10ml water and was kept for 24 hrs to obtain the extract. After that the mixture was filtered using Whatman filter paper and the liquid obtained was dried in hot air oven in order to obtain dry extract. Then 3mg of both dried extracts was dissolved in 1ml water in microcentrifuge tubes and were sonicated for 15min, then filtered with 0.2µm 13mm nylon membrane filters before injecting them in the machine.[13]

P reparation of Mobile Phase :

A Mixture Methanol & ACN (70:30) was prepared for the analysis. The prepared mixture was Then degassed in an ultra sonicator for 15 min.

Chromatographic Condition

Table 1: Chromatographic Conditions used for analysis of *Agaricus bisporus* powder sample

S.No.	Parameters	Condition
1	Stationary phase	Agilent TC-C18(2),4.6x250mm,5um
2	Mobile phase	Methanol: ACN (70:30)
3	Detection Wavelength	254 nm
4	Flow rate	1 ml/min
5	Injection volume	20 µl
6	Temperature	Ambient
7	LC System	Agilent test system and OpenLab CDS2

Instruments/reagents details

HPLC: G4288C 1220 Infinity II Gradient LC System VL
Membrane filters: Membrane filter 0.2 µm, PTFE GL 14
Sonicator: Ultrasonic Bath Sonicator ATS-1
HPLC grade solvents: Methanol & Acetonitrile
Weighing machine: Digital Mettler Analytical Balance Mettler Me204

RESULTS

Qualitative analysis of *Agaricus bisporous*

Table 2 : Qualitative analysis results for analysis of *Agaricus bisporus*powder sample

S.No.	Test	*Agaricus bisporous* (Aqueous extracts)
1	Alkaloids	Present
2	Terpenoids	Present
3	Flavonoids	Present
4	Steroid	Present
5	Saponin	Present
6	Tannin	Present
7	phenol	Present

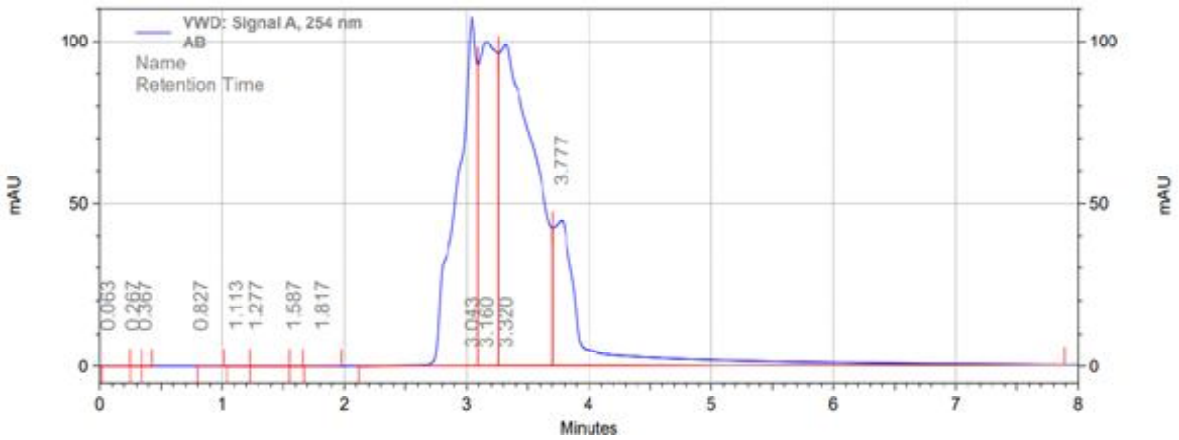

Fig. 1. Chromatogram of Agaricus bisporus sample at 254 nm

Table 3: Retention time of qualitative analysis of *Agaricus bisporus*given powder samples

Retention Time	Area	Area %	Height	Height %
0.063	1602	0.00	144	0.00
0.267	618	0.00	122	0.00
0.367	281	0.00	85	0.00
0.827	222	0.00	26	0.00
1.113	492	0.00	54	0.00
1.277	1055	0.00	99	0.00
1.587	94	0.00	30	0.00
1.817	550	0.00	71	0.00
3.043	20208076	24.59	1805130	30.70
3.160	16640915	20.25	1674424	28.47
3.320	32720982	39.82	1655922	28.16
3.777	12596591	15.33	744313	12.66
Totals	82171478	100.00	5880420	100.00

DISCUSSION

In the present study, the qualitative phytochemical screening of *Agaricus bisporus* revealed the presence of notable secondary metabolites.Flavonoids, phenols, saponins, glycosides, and tannins are examples of phytochemical substances that are thought to be important secondary metabolites in mushrooms.In our investigation, initial phytochemical Several active constituents are found by screening. These active metabolites are widely recognised for their ability to treat a variety of human conditions, including menstrual disorders, chronic eczema, diuretic choleretic, spasmodic, diarrhoea, and dysentery.

Because of their incredible chemical

diversity and immense therapeutic potential, natural products are regarded as a rich store of bioactive components[18]. They include substances originating from plants, fungi, and microorganisms, as well as semi-synthetic ones. These compounds have a broad range of medicinal applications, including anticancer, antibacterial, and antioxidant properties[19]. Secondary metabolites of mushrooms, such as phenolic compounds, alkaloids, and glycosides, serve important nutritional and medicinal roles. Extensive research and long-standing traditional use have shown that certain mushrooms contain essential physiologically active chemicals that are useful due to their antioxidant properties. Among these, phenols, flavonoids, tannins, alkaloids, and glycosides are known for their antioxidant and antibacterial properties. The existence of various bioactive chemicals is revealed by our HPLC results according to the molecular formula, retention time-molecular weight, and peak area. During the current analysis, a number of chemicals were found in A. bisporus; the main phytochemical components found may be the cause of its antioxidant, antidiabetic, and antibacterial qualities. According to the HPLC results, ergothioneine and gallic acid were discovered in the chromatogram of the A. bisporus extracts at a retention time of 3.320 and 3.777 minutes, respectively, as evidenced by the study of Nitthikan, N, *et al*. 2022[14]. Figure 1 displays the amounts of gallic acid and ergothioneine in A. bisporus extracts. Remarkably, ergothioneine has been shown to possess anti-inflammatory and antioxidant properties[15]. Serine, ergothioneine, glutamic acid, and alanine are among the amino acids that are abundant in A. bisporus. High amounts of ergothioneine have been found in the fruiting body of A. bisporus[16]. Studies have demonstrated the anti-inflammatory, anti-aging, and antioxidant properties of phenolic substances such gallic acid, ferulic acid, and caffeic acid. Furthermore, a prior work[17] identified gallic acid as the primary phenolic acid in *A. bisporus* extracts. Thus, based on these findings, we can infer that gallic acid and ergothioneine are the two main, abundant bioactive substances in *A. bisporus* extracts that are connected to the extracts' biological activity. More thorough research, including in vitro experiments, is required in light of the preliminary findings. Thus, the mushroom in this study may have a variety of medicinal importance and nutritional value.

CONCLUSION

From the chemical examination it can be concluded that *Agaricus bisporus* has highly nutritional value and medicinal importance, incredible chemical diversity, immense therapeutic potential and rich store of bioactive components. *Agaricus bisporus* have broad range of medicinal application including anticancer, antibactertial and antioxidant properties.

Scope of future work in the research field :

Scope of future work of white button mushroom is very bright and helped in research field. In future we study about blood sugar lowering effect i.e. **Antidiabetic Activity, Antimicrobial Activity**, **Antioxidant Property** etc. of white button mushroom.

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