JSRT JOURNAL

PAGES: 96-105
18/09/25

JOURNAL OF SCIENTIFIC RESEARCH AND TECHNOLOGY VOLUME-3 ISSUE-9 SEPTEMBER

Evaluation Of Phytoconstituents In Edible White Button Mushroom *Agaricus Bisporus.*

Dr Yasmin Bano and Heba Ashraf*

Oriental College, Patliputra University, Patna.

*Corresponding author: hebapatna@gmail.com

ABSTRACT

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 indicate 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, the mushroom employed in this study may find application in the pharmaceutical business for a variety of medical purposes.

Keywords: *Agaricus bisporus*, HPLC analysis, Antioxidant, phytochemicals, Phenolic compounds.

INTRODUCTION

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 fewer adverse effects. Plants that have been selected for medical use for 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 different 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 several bioactive compounds that have shown 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*.

MATERIALS AND METHODS

Preparation of plant extracts

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 the refrigerator when not in use.

Qualitative Phytochemical Screening of *Agaricus bisporus*

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 10 mg of crude extract added 1 mL of lead acetate. Formation of precipitate indicates the presence of phenolic compounds.

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

Test for Saponins: To 1 mL of extract added 5 mL of distilled water was added, shaken well, and the formation of foam indicates the presence of saponins.

Test for Flavonoids: A few drops of 10% lead acetate were added to 10 mg of plant extract, yellow colour precipitate indicates the presence of Flavonoids. A few drops of 10% NaOH are 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. The upper layer turns red, and the H_2SO_4 layer shows a yellow colour, indicating the presence of steroids.

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

Preparation of Sample for HPLC analysis

For analysis of *Agaricus bisporus* powder sample, extraction was done using the 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 a hot air oven to obtain the dry extract. Then 3mg of both dried extracts was dissolved in 1ml water in microcentrifuge tubes and was sonicated for 15min, then filtered with 0.2µm 13mm nylon membrane filters before injecting them in the machine. [13]

Preparation of Mobile Phase:

A Mixture of Methanol & ACN (70:30) was prepared for the analysis. The prepared mixture was then degassed in an ultrasonicator for 15 min.

Chromatographic Conditions:

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

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

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 bisporus*

S.No.	Test	*Agaricus bisporous* (Aqueous extracts)
1	Alkaloids	+
2	Terpenoids	+
3	Flavonoids	+
4	Tannin	+
5	Saponin	+
6	Phenol	+
7	Steroids	+

Presence (+) Absence (-)

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

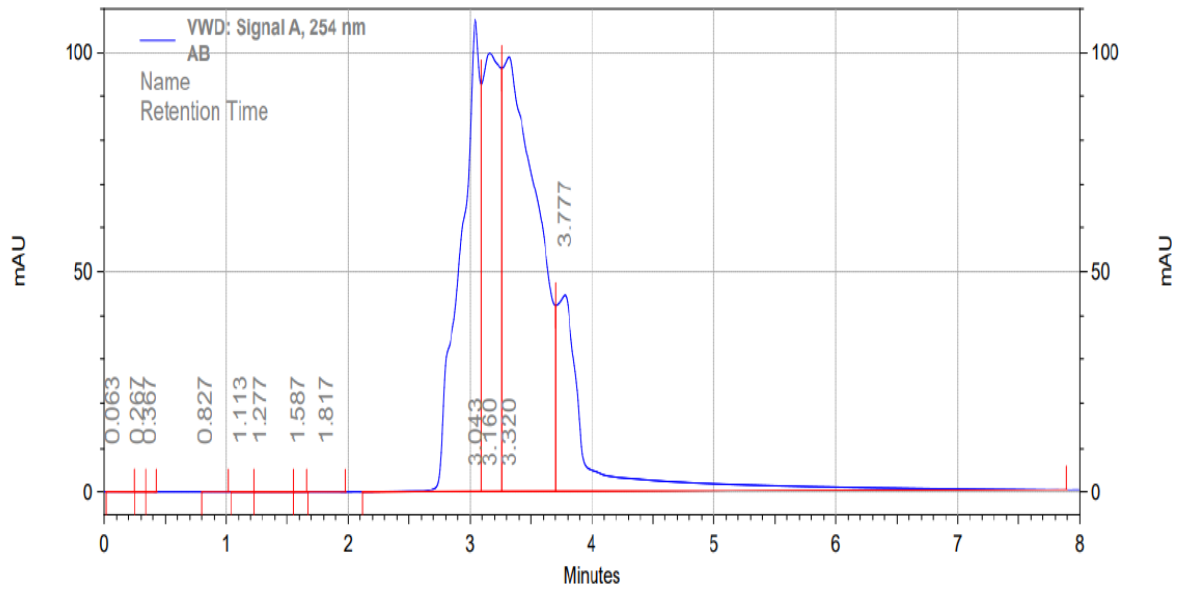

Fig 1: ***Chromatogram of Agaricus bisporus sample at 254 nm***

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**

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

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, the 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, several 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]. 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* [15, 16]. Studies have demonstrated the anti-inflammatory, anti-ageing, and antioxidant properties of phenolic substances such as gallic acid, ferulic acid, and caffeic acid. Furthermore, a prior work identified gallic acid as the primary phenolic acid in *A. bisporus* extracts [17] . 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 medical applications in the pharmaceutical business.

REFERENCES

[1] Dewick, P.M. 1996. Tumor inhibition from plants: Tease and Evans.

[2] Phillipson, J.D., Wright, C.W. 1996. Plants With Antiprotozoal Activity : Tease and Evans, Pharmacognosy, 14th edn., WB Saunders Company, London, pp. 612.

[3] Arunkumar, S., Muthuselvam . 2009. Analysis of phytochemical constituents and antimicrobial activities of aloevera L. against clinical pathogens. World J. Agril. Sc., 5(5): 572 576.

[4] Edoga, H.O., Okwu, D.E., Mbaebie, B.O. 2005. Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4(7): 685-688.

[5] Mann, J.1978. Secondary Metabolism. Oxford University press, London, pp. 154.

[6] Vasu, K., Goud, J.V., Suryam, A., Singara, Chary, M.A. 2009. Biomolecular and phytochemical analyses of three aquatic angiosperms. Afr. J. Microbiol. Res., 3(8):418-421.

[7] Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 564-582.

[8] Criagg, G.M., David, J.N. 2001. Natural product drug discovery in the next millennium. J. Pharm. Biol., 39: 8-17.

[9] Mojab, F., Kamalinejad, M., Ghaderi, N., Vanidipour, H.R. 2003. Phytochemicals screening of some species of Iranian plants. Iran. J. Pharm. Res., 3: 77-82.

[10] Parekh, J., Chanda, S. 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res., 10: 175-181.

[11] Parekh, J., Chanda, S. 2008. Phytochemicals screening of some plants from western region of India. Plant Arch., 8: 657-662.

[12] JagadishL. K., Krishnan V. V., Shenbhagaraman R., & Kaviyarasan V. Comparitive study on the antioxidant, anticancer and antimicrobial property of Agaricus bisporus (JE Lange) Imbach before and after boiling. Afr. J. Biotechnol. 2009; 8:4.

[13] Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. Q2 (R1), 1(20), 0

[14] Nitthikan, N.; Leelapornpisid, P.; Naksuriya, O.; Intasai, N.; Kiattisin, K. Potential and Alternative Bioactive Compounds from Brown Agaricus bisporus Mushroom Extracts for Xerosis Treatment. Sci. Pharm. 2022, 90, 59. https://doi.org/10.3390/scipharm 90040059

[15] Cheung, B.; Kwan, M.; Chan, R.; Sea, M.; Woo, J. Potential of Asian natural products for health in aging. Mol. Basis Nutr. Aging 2016, 2016, 659–676

[16] Muszynska, B.; Kala, K.; Rojowski, J.; Grzywacz, A.; Opoka, W. Composition and biological properties of Agaricus bisporus fruiting bodies-a review. Pol. J. Food Nutr. Sci. 2017, 67, 173–181.

[17] Ramos, M.; Burgos, N.; Barnard, A.; Evans, G.; Preece, J.; Graz, M.; Ruthes, A.C.; Jiménez-Quero, A.; Martínez-Abad, A.; Vilaplana, F. Agaricus bisporus and its by-products as a source of valuable extracts and bioactive compounds. Food Chem. 2019, 292, 176–187.

[18] Huang M., Lu J. J., & Ding J. Natural products in cancer therapy: Past, present and future. Nat. prod.Bioprospect. 2021;11:5-13.

[19] HarveyA. L. Natural products in drug discovery. Drug discov. Today. 2008;13 suppl. 9 20:894-901.