International Journal of Multidisciplinary Research and Growth Evaluation.

Phytochemical Screening of *Agaricus bisporus* with Medicinal Plants Supplementation

Swati Jha [1*], Dr. Arun Kumar [2]
[1] Research Scholar, Department of Botany, Ranchi University, Jharkhand, India
[2] Associate Professor (Retd.), Former Head and Dean, Department of Botany, Ranchi University, Jharkhand, India

* Corresponding Author: **Swati Jha**

Article Info

ISSN (online): 2582-7138
Volume: 06
Issue: 04
July - August 2025
Received: 09-06-2025
Accepted: 10-07-2025
Published: 05-08-2025
Page No: 1186-1189

Keywords: *Agaricus bisporus*, *Zingiber officinale*, *Curcuma longa*, Aqueous Extract, Phyto-Constituents

Abstract
This work has explicitly investigated the phytochemicals in *Agaricus bisporus* when cultivated with *Zingiber officinale* and *Curcuma longa* parts as botanical supplementations. Here, the phytochemical screening of with aqueous extracts of the mushrooms has revealed the presence of phyto-constituents like carbohydrates, proteins, alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides, steroids and saponins.

Introduction
The world has reached to a population of 7 billion and still growing leading to shortage of food resulting in health deterioration in humans [1]. There has been constant shortage of food and shelter. Speaking about food, people definitely need proper nourishment to live a healthy life and especially vegetarians who do not take animal protein and for making proper shelter, there has been constant deforestation leading to shortage of plants and in turn plant protein. Here, comes a voice from the wild speaking about source of stored protein named mushrooms. These edible fungi can fulfill the nutritional demands of human beings [2, 3]. *Agaricus bisporus* cultivation contributes to about 40% of all mushrooms grown [4]. The common name of this mushroom is white button mushroom and is widely used in the treatment of cerebral strokes and cancers reported [5]. *Agaricus bisporus* is most popular variety in India with high prices [6]. This mushroom has a credible sources of micro elements like sodium, potassium, phosphorus, antioxidants, conjugated linoleic acid etc [7].
The extract of this mushroom can be probably utilized in Alzheimer's disease as it has butyryl-cholinesterase and acytyl-cholinesterase inhibition activity [8]. *Agaricus bisporus* extracts and its phytochemical plant compounds are used as anti-cancerous, anti-inflammatory, antioxidants against many diseases like diabetes mellitus, coronary heart diseases, immune system disorders, fungal and bacterial infections etc [9, 15]. Three essential polysaccharides are present in *Agaricus bisporus* that is alpha-glucan, beta glucan and galactomannan [16] and mainly fatty acids which are linoleic acid, palmitic acid, and stearic acid [17]. The main aim of this research is to screen out the phyto-constituents of *Agaricus bisporus* when cultivated with *Curcuma longa* and *Zingiber officinale*.

Materials and Methods
Cultivation of Mushrooms
The cultivation process was conducted in Birsa Agriculture University, Kanke Ranchi, Jharkhand. Process includes the usual four steps- composting, spawning, casing and harvesting. Two botanicals were taken for supplementation that is, *Zingiber officinale* and *Curcuma longa*. Now, considering the economical benefits and production it was observed that all the 4% supplementation gave good results in comparison to 2% and in some cases both were almost equal on the average. So, the further phytochemical and other deep studies were restricted to 4% supplements.

Fig 1

Classification

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	Agaricaceae
Genus	*Agaricus*
Species	*Agaricus bisporus*

Medicinal Plants Supplementation

The rhizome of both the plants were taken for the supplementation purpose. They were washed. Cut into thin slices, dried and powered. They were incorporated in the casing material in certain percentages that is, 2% and 4% each. Untreated bags were kept as controls. All the bags were replicated thrice.

Then following the usual time and method, the fruiting was harvested from the bags and taken for preliminary phytochemical screening.

Drying of Mushrooms

Freshly harvested mushrooms were thoroughly washed to remove impurities, cut into thin slices and dried in shade for 3-4 weeks. Then, they were crushed and powered in a mortar and pestle.

Preparation of Aqueous Extract

Powder was dissolved in distilled water in the ratio of 1:10. After that it was boiled in a water bath at a controlled temperature. Then, the preliminary qualitative phytochemical screening was carried on-

The standard methods were used for the qualitative screening of white button mushrooms [18, 20].

1) Test for Carbohydrates

a) Benedict's test

1 ml extract + 1 ml Benedict's reagent

This gave a bluish green color that indicated the presen of carbohydrates.

2) Test for Proteins

a) Million's test

1 ml extract + 5-6 drops of Million's reagent

A white precipitate was obtained that indicated the presence of proteins.

3) Test for Alkaloids

a) Dragendroff's test

1 ml extract + 1 ml HCl + 1 ml Dragendroff's reagent

A red to turbid orange color indicated the presence of alkaloids.

4) Test for Flavonoids

a) Lead acetate test

1 ml extract + 2 ml distilled water + 3 ml of 10% lead acetate

A bulky white precipitate indicated the presence of flavonoid.

5) Test for Anthocyanine

a) Hydrochloric test

1 ml extract + 5 ml dilute HCl

Pink color appearance indicated the presence of anthocyanine.

6) Test for Phlobotannins

1 ml extract + few drops of Dilute HCl

Appearance of red precipitate indicates the presence of phlobotannin.

7) Test for Glycosides

a) Keller-Killiani Test

5 ml extract + 2 ml glacial CH_3COOH + 1 drop of ferric chloride + 1 ml Conc. H_2SO_4

Formation of a brown ring indicates the presence of glycosides.

8) Test for Phenols and Tannins

a) Ferric chloride test

1 ml extract + 1 ml of 5% $FeCl_3$

Blue green color appears.

9) Test for Terpenoids

a) 2 ml extract + 2 ml chloroform + 3 ml Conc.H_2SO_4

Appearance of reddish brown color indicates the presence of terpenoides.

10) Test for Steroids

a) 2 ml extract + 5 ml chloroform + 3 ml Conc.H_2SO_4.

Upper red layer, lower yellow with green fluorescence were observed.

11) Test for Saponins

a) Foam test

2 ml extract shaken vigorously

There was a development of foam that indicated the presence of saponins.

Table 1: Phytochemical analysis of *Agaricus bisporus* with supplementations.

Serial no.	Test	M+Z	M+C	NS	Color indication
1	Carbohydrate				
a	Benedict's Test	+	+	+	Dark Green
2	Proteins				
a	Million's Test	++	++	++	White ppt.
3	Alkaloids				
a	Dragendroff's Test	+	++	+	Red to turbid orange color
4	Flavonoids				
a	Lead acetate Test	+ +	++	+	Bulky white ppt.
5	Anthocyanine				
a	Hydrochloric Test	–	–	–	Pale pink coloration
6	Glycosides				
a	Keller-Killiani Test	++	+	–	A Brown ring formation
7	Phlobotannins				
a	Phlobotannin test	–	–	–	Red ppt.
8	Phenols and Tannins				
a	Ferric Chloride Test	+++	++	++	Blue green to blue black coloration
9	Terpenoids				
a	Salkowski Test	++	+++	+	Reddish Brown color at interface
10	Steroids				
a	Steroidal test	+	++	+	Upper layer red and lower layer yellow
11	Saponins				
a	Foam Test	+	+	+	Development of foam

(+) = present, (++) = moderately present, (+++) = highly present, (-) = absent
M+Z=Mushroom extract with *Zingiber* supplementation.
M+C=Mushroom extract with *Curcuma* supplementation.
NS = Non-supplemented mushroom extract.

Results and Discussion

The present study was just a preliminary phytochemical screening that reflected the presence of many important phyto-constituents. *Agaricus bisporus* is in itself a source of a variety of bioactive compounds that imparts them antimicrobial properties and a study also showed comparatively high concentration of proteins and tannins than other phyto- constituents [21]. Many supplementations have been done in mushroom composts. Here, in this work medicinal plant supplemented mushrooms have been taken into account for detection of phytochemicals. The preliminary observations were based on visual intensity comparison where it was found that flavonoids, steroids, terpenoids, glycosides, tannins and phenols were comparatively slightly higher in intensity in mushroom extract with supplementations than non-supplemented case and in no supplement case glycosides were very light or not traced. Carbohydrates, proteins and saponins are also present. While, anthocyanins were absent and also phlobotannins were also not visible clearly. Phytochemical components in oyster mushroom supplemented with medicinal plants were more than in control [22].

Conclusion

Mushrooms are full package of important bio compounds with all important properties. Various modifications in the form of supplementations are done to enhance their different nutritional and phytochemical aspects .They are packed with different phytochemicals like alkaloids, flavonoids, steroids, terpenoids, glycosides, phenols, tannins, proteins, carbohydrates and saponins with an increased intensity of presence of steroids, alkaloids, terpenoids, tannins and phenols in supplemented extracts than non-supplemented ones.

Acknowledgement

The first author is grateful to the University Department of Botany, Ranchi University, Ranchi for providing all the facilities. The author is also grateful to our guide and Associate Professor Dr. Arun Kumar (Retd.).

Conflict of Interest

The authors report no conflict of interest.

Reference

1. Livi Bacci M. A Concise History of World Population. 5th ed. Malden, MA: Wiley-Blackwell; 2012. p. 50.
2. Hawksworth DL. The fungal dimension of biodiversity: Magnitude, significance, and conservation. Mycol Res. 1991;95:641-55.
3. Chang ST. The world mushroom industry: Trends and technological development. Int J Med Mushrooms. 2006;8(4):297-314.
4. Thakur MP, Singh HK. Advances in the cultivation technology of tropical mushrooms in India. JNKVV Res J. 2014;48:120-35.
5. HealthBlog247. Some benefits of *Agaricus bisporus* extract [Internet]. [cited 2017 Dec 25]. Available from: http://www.healthblog247.com/some-benefits-of-agaricus-bisporus-extract/
6. Mehta BK, Jain SK, Sharma GP, Doshi A, Jain HK. Cultivation of button mushroom and its processing: A techno-economic feasibility. Int J Adv Biotechnol Res. 2011;2:201-7.
7. Shiuan C, Sheryl P, Gene H, Sharon K, Jingjing Y, *et al*. Breast cancer prevention with phytochemicals in mushrooms. Proc Amer Assoc Cancer Res. 2005;46:5186.
8. Arianne V, Julian Renato GR, Fumio E. Agro-industrial waste conversion into medicinal mushroom cultivation. Ref Module Earth Syst Environ Sci. 2018.
9. Winer EP, Hudis C, Burstein HJ. American Society of

Clinical Oncology technology assessment of the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor-positive breast cancer: Status report. J Clin Oncol. 2002;20(15):3317-27.
10. Dhamodharan G, Mirunalini S. A novel medicinal characterization of *Agaricus bisporus* (white button mushroom). Pharmacol Online. 2010;2:456-63.
11. Volman JJ, Mensink RP, van Griensven LJ, Plat J. Effects of α-glucans from *Agaricus bisporus* on ex vivo cytokine production by LPS and PHA-stimulated PBMCs; a placebo-controlled study in slightly hypercholesterolemic subjects. Eur J Clin Nutr. 2010;64:720-6.
12. Ozturk M, Duru ME, Kivrak S, Dogan NM, Turkoglu A, *et al*. *In vitro* antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. Food Chem Toxicol. 2011;49(6):1353-60.
13. Mao Y, Mao J, Meng X. Extraction optimization and bioactivity of exopolysaccharides from *Agaricus bisporus*. Carbohydr Polym. 2013;92(2):1602-7.
14. Ghahremani-Majd H, Dashti F. Chemical composition and antioxidant properties of cultivated button mushrooms (*Agaricus bisporus*). Hortic Environ Biotechnol. 2015;56:376-82.
15. Ndungutse V, Mereddy R, Sultanbawa Y. Bioactive properties of mushroom (*Agaricus bisporus*) stipe extracts. J Food Process Preserv. 2015;39:2225-33.
16. Smiderle FR, Ruthes AC, Van Arkel J, Chanput W, Lacomin M, *et al*. Polysaccharides from *Agaricus bisporus* and Agaricus brasiliensis show similarities in their structures and their immunomodulatory effects on human monocytic THP-1 cells. BMC Complement Altern Med. 2011;11:58.
17. Shao S, Hernandez M, Kramer JKG, Rinke DL, Tsao R. Ergosterol profiles, fatty acid composition, and antioxidant activities of button mushrooms as affected by tissue part and developmental stage. J Agric Food Chem. 2010;58(22):11616-25.
18. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books; 1993. p. 289.
19. Trease GE, Evans WC. Pharmacognosy. 11th ed. London: Baillière Tindall; 1978. p. 115-222.
20. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998. p. 114-8.
21. Lall R, Singh P, Jha A, Gupta V, Nagar S. Comparative study of nutritional, antimicrobial and antioxidant properties of Pleurotus ostreatus and *Agaricus bisporus*. J Pharmacogn Phytochem. 2017;SPI:760-3.
22. Nosh SV, Tacham WN, Ngwang MV, Kinge TR. The effect of substrates on the growth, yield, nutritional and phytochemical components of Pleurotus ostreatus supplemented with four medicinal plants. Afr J Biotechnol. 2022;21(6):292-304.