IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

IN-VITRO ASSESSMENT OF ANTIBACTERIAL, ANTIOXIDANT AND TOXICITY STUDIES OF AGARICUS BISPORUS WITH MUSHROOM COFFEE FORMULATION

Mr.Ganu.C.Gavade, Ms.Rutuja.N. Patil, Ms.Nikita.B.Dalavi, Ms.Snehal.S. Salokhe

Department of Pharmacognosy,

Rani Chennamma college of Pharmacy, Belagavi, Karnataka, India

ABSTRACT

Development of some novel value added products were undertaken from the fresh-dried mushroom [Agaricus bisporus]. In this context, an attempt was made to evaluate antibacterial, antioxidant and cytotoxic activity of ethanolic extract of Agaricus bisporus. Dried coarse powder of mushroom was subjected to extraction with ethanol by cold maceration and then evaporated using electric water bath. Stock solution of the extract was prepared using distilled water. Preliminary phytochemical screening was carried out. The extract was evaluated for Antibacterial activity by agar well diffusion method, antioxidant activity by DPPH assay and cytotoxicity study by BSL assay. And along with these studies, attempt was made to prepare mushroom coffee.

KEY WORDS

Agarius bisporus, 0.2 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Brine Shrimp Lethality assay (BSL), agar well diffusion, and Mushroom coffee.

INTRODUCTION

Fungi are vital to numerous industrial and household processes, especially producing cheese, beer, wine, coffee and breads. The remarkable medicinal and nutritional values of mushroom have increased their consumption.(1) The button mushroom, Agaricus bisporus, is the most important commercially cultivated mushroom in the world. However, as compared to other mushrooms recognized as medicinal mushrooms, fewer studies have been undertaken on various activities of Agaricus bisporus.(2)

Agaricus bisporus (mushroom) it is also called as button mushroom and belongs to Agaricaceae family are considered as potential source of many essential nutrient and therapeutic bioactive compound.[1] The rich nutrient like carbohydrate, proteins, lipids, fibers, minerals and vitamins are present in this mushroom as famous healthy food. Moreover, because of the presence of some active ingredient, such as polysaccharide, lipopolysaccharide, essential amino acids, peptides, glycoprotein, nucleoside, triterpenoids, lectins, fatty acids and their derivatives.[3] These mushroom have been reported to have a number of studies on the biological activities and chemical constituents of A. bisporus. White button mushroom lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemia. The main purpose of the selection of this Agaricus bisporus, there was no systematic and scientific investigation has been carried out so far. Thus, the present study focused on phytochemical evaluation of the ethanolic extract of Agaricus bisporus. [4]

Therefore, the objective of this study was to investigate the antimicrobial activity of coffee extracts and of coffee bioactive compounds against enterobacteria, Ground coffee was analyzed for color, and the extracts were characterized according to pH and levels of selected chemical compounds. [8] Therefore, this study is conducted to access antibacterial, antioxidant, and toxicity study activities of A.bisporus. As a result, various Indian medicinal plants were chosen based on Ayurvedic text and folklore claims, and their cytotoxicity was examined using the BSL bioassay. [9]

MATERIAL AND METHOD

Collection of Plant

The selected plant, Agaricus Bisporus were collected from the nearby cold storage, Belagavi district (Karnataka) during august 2022.

List of Chemicals

Sr.No	Name	Manufacturer
1	Distilled Water	RCCP , Belagaum
2	95% Ethanol	RCCP, Belagaum
3	Nutrient Agar	RCCP ,Belagaum
4	Standard Drug- Beta glucan	Medplus .,Belagaum
5	0.2mm2,2di-phenyl-1-picrylhydrazyl(DPPH)	Maratha Mandals Central Research Laboratory, Belagaum.
6	0.1m tris HCL(pH 7.4)	Maratha Mandals Central Research Laboratory, Belagaum.
7	Standard: Vit.C as antioxidant	Medplus .,Belagaum
8	Mushroom Powder	RCCP , Belagaum
9	Coffee Powder	Local market, Belgaum

METHOD OF PREPRATION

a. Preparation of extract

1. Extraction of Agaricus Bisporus

Fresh Fruiting bodies were dried in shed condition. Dried material (75 gram) was pulverized in blender to get course powder. Soaked in 450 ml of ethanol in Conical Flask and the flask was covered With Aluminum Foil.6-7 days with frequent shaking and extract was Filtered through Whatman filter paper No.1. Evaporation and collection of extract. [10]

2. Maceration

The fresh fruiting bodies were dried in shed condition and the dried material (75 gram) was pulverized in a blender to get a course powder and soaked separately in 450 ml of ethanol and in Erlenmeyer flask. The flasks were covered with Aluminum Foil and allow standing for 7 days for extraction. This extract were filtered through Whatman filter paper No.1 and evaporated at 40 degree celeries using electrical evaporated .the extract were collected and stock solution of conc. 10 mg/ml was prepared. [10]

Preliminary Phytochemical Screening:

The component Such as Agaricus biporus extract has been used for phytochemical test to assess qualitative chemical composition by standard method.

1. Test for alkaloids: [16,17]

A sample of 0.5gm To 0.6 gram was mixed in 8ml of 1% HCl ,heated and filtered .2ml of filtrate were treated with the following reagent after which it was observed weather alkaloid present or absent with turbulence formation of precipitate .

a.Mayers Test - A few drops of Mayer's reagent in 2-3 ml of filtrate indicate a precipitate of cream.

b.Dragendroff's Test - A few drop of the drgendroff's reagent show reddish brown precipitate in 2-3ml.

2. Test for Carbohydrates

Molisch's Test (General Test): For 2-3 ml of aqueous sample solution, mix, shake and compress a few drops of alpha-naphthol solution. H_2SO_4 from the sides of the test tube. The formation of a violet colour at the junction of the two fluids indicates the presence of carbohydrates.

Benedicts test: The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

3. Test for Flavonoids

Shinoda test: In a sample of dried powder, add 5% 95% ethanol, some Conc. drop. HCI and 0.5 g magnesium turnings. The presence of orange, pink and red to purple confirms the presence of flavonoids

Ferric chloride test: Few drops of neutral ferric chloride solutions are added to little quantity of alcoholic extract. A blackish green colour produced indicates the Phenolic nucleus.

4. Test for Tannins

Ferric chloride test: Few drops of neutral ferric chloride solutions are added to little quantity of alcoholic extract. A blackish green colour produced indicates the phenolic nucleus.

5 .Test for Steroids

Salkowski test: 0.5 g samples were carefully mixed in 2 ml of chloroform and 3 ml of concentrate (H_2SO_4). A layer of greenish yellow or reddish brown has been formed at the interface indicating a positive result for the presence of steroids.

7 .Tests for Proteins:

Millon's test: Treat the test sample with a few drops of Millon's reagents, when it is heated, a white precipitated form that turns brick red or disappears indicating the presence of protein.

Screening Of Extract Of Agaricus Bisporus For Antibacterial Activity [10]

Antibacterial activity of ethanolic extract of Agaricus bisporus was done using agar well diffusion method.

Nutrient agar media was used throughout the investigation the medium was autoclaved at 121.6°C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours.100 microlitre of bacterial suspension was spread on 2 nutrient agar plate.2 agar wells were prepared with the help of sterilized stainless steel borer in both Petri plates.1 well in each Petri plates were loaded with prepared extract of Agaricus bisporus of concentration 10 mg/ml. and the other wells in each Petri plates were loaded with standard drug in a concentration 10mg/ml.The plates were incubated at 37±2°C for 24 hours in the incubation chamber. The zone of growth inhibition was determined.Screening Of Antioxidant Activity Of Ethanolic Extract Of Agaricus Bisporus [10]

Preparation of extract:

Weigh 10mg of extract and dissolve in 1ml of DMSO.

Procedure:

Take test tubes and label as blank control and test. Add the reagents as follows Blank 600microlitre ethanol + 400microlitre Tris HCL Control 100microlitre ethanol + 400microlitre Tris HCL + 500microlitre DPPH solution Test 100microlitre sample + 400microlitre Tris HCL + 500microlitre DPPH solution Mix all the test tubes and keep in dark for 30minutes Read the absorbance at 490nm.

Calculation: [6]

As -sample O.D (optical density)

Ac -control O.D

$$\text{Inhibition ratio\%} = \frac{AC-AS}{AC} X100$$

Brine Shrimp Bioassay:[25]

Cytotoxicity of the all-plant extract and fractions were determined by BSL bioassay. The brine shrimp eggs were procured and filtered sea water (11) was added into the hatching chamber; sprinkle shrimp eggs (50 mg). Allow 2 days (48 h) for the shrimp to hatch and mature as nauplii (hatched shrimp). As the nauplii are phototropic in nature they will move towards smaller illuminated compartment through the holes made on compartment divider. Samples and standard were prepared in vials for testing to get final concentrations 1, 10, 100, 1000µg/ml: all the samples and standard were prepared in triplicate.

Preparation of Stock solution: [25]

The standard stock solution was prepared by dissolving 10 mg of each in 10 ml in methanol to obtain 1000 µg/ml which becomes the main stock solution. 1 ml was removed from the primary stock solution and diluted to 10 ml to give 100 µg/ml that becomes the secondary stock solution. As the solution is sensitive to light, the solutions were prepared in an amber-colored volumetric flask.

Nauplii were drawn from the hatching chamber using bulb pipette against light background and exactly 10 shrimps were transferred to each test tubes. Then drug samples were added to each test tube that was previously marked in triplicate for each extract/fraction. The sea water was added to each test tube to make the volume up to 5 ml. A drop of dry yeast suspension (3 mg in 5 ml sea water) was added to each test tube as a food for shrimps. The test tubes were maintained under illumination. After 24 h, number of survivors were counted and recorded and the lethal concentration (LC50) values were calculated by means of Statistical Package for the Social Sciences (SPSS)-20 software. The fractions with LC50 values < 100 ppm were selected for further studies.

Formulation of mushroom coffee-[14]

SR.NO	INGREDIENT NAME	QUANTITY
1	Mushroom Powder	0.5gm
2	Coffee Powder	1gm
3	Sugar	12.50gm
4	Milk	100ml
5	Water	q.s

RESULTS AND DISCUSSION –

EXTRACTION:

Extraction was performed on Agaricus bisporus by maceration process with 95% ethanol for 7 days.75g of dried mushroom powder in 450ml of ethanol was subjected for extraction.

Then extract was subjected to evaporation using electrical water bath, and 5.75g of dried extract was obtained, then extracts was used for various pharmacognostic and in-vitro screenings.

PRELIMINARY PHYTOCHEMICAL SCREENING:

Phytochemical screening was performed to determine the various phytoconstituents present in Agaricus bisporus.

Sr.no	Phytochemical test	Tests	Observation	Inference
1	Glycosides	Baljet test	Orange colour	+
		Legal test	Red colour	+
2	Flavonoids	Shinoda test	Magenta Colour	+
		Ferric chloride test	Blackish Green Colour	+
3	Tannins	Ferric chloride test	Blackish Green Colour	+
4	Carbohydrates	Molish test	Reddish violet colour	+
		Benedicts test	Brown ppt	+
5	Alkaloids	Mayers test	No Ppt	–
		Dragaondorffs test	No Ppt	–
6	Steroids	Salwoski test	Lower layer turns red	+

PRELIMINARY PHYTOCHEMICAL TEST:

Phytoconstituents	Observation
Glycosides	+
Flavonoids	+
Tannins	+
Carbohydrates	+
Steroid	+
Protein	+

The Extract was found to content glycosides, flavonoids, tannins, carbohydrate, steroids and protein. The result of the phytochemical screening are express in below;

SCREENING OF ANTIBACTERIAL ACTIVITY OF AGARICUS BISPORUS:

The ethanolic extract of Agaricus bisporus was screened for gram negative bacteria by agar well diffusion method. Stock solution was prepared by making a concentration to 10mg/ml.The result of zone of inhibition of test sample and standard drug are as follows,

Sample	Zone of Inhibition
Test	1.7cm
Standard	2.1cm

SCREENING OF ANTIOXIDANT ACTIVITY OF AGARICUS BISPORUS:

The ethanolic extract of Agaricus bisporus was screened for antioxidant activity by DPPH assay method. The absorbance readings of test, control, and blank are as follows.

Sample	Absorbance (nm)
Standard	0.270
Control	1.543
Test	0.539
Blank	0.144

The following formula was used to calculate % inhibition of test, control standard, and blank,

$$\text{Inhibition ratio\%} = \frac{\text{AC}-\text{AS}}{AC}\text{X}100$$

Where,

Ac- control OD (optical density) and As- sample OD

The results of inhibition ratio % of standard and test are as below

Sample	**Inhibition ratio %**
Standard	82.50%
Test	65.06%

CYTOTOXICITY STUDY OF AGARICUS BISPORUS:

Cytotoxicity effect of the ethanolic extract of Agaricus Bisporus by BSL assay:

Cytotoxicity effect of ethanolic extract of Agaricus Bisporus was done by BSL assay. The extract showed significant cytotoxic activity, the extract was almost 100% lethal to brine shrimp at the concentration 1000µg/ml.

Result is expressed as mean ± standard. Error of mean. Cytotoxicity (mean% death after 24 hrs with LC50 values) of the extracts was compared with those of the control.

Plant	Extract	Mean % Death after 24hrs (concentration in µg/ml) LC 50 (PPM)				
		1	10	100	1000	LC 50
Agaricus bisporus	Ethanolic extract of Agaricus bisporus	2.3 ±0.2	44.40 ± 0.33	87.70 ± 0.22	100	25.2

CONCLUSION

The present work demonstrates, the study conducted on microscopic, macroscopic, preliminary phytoconstituent screening, antibacterial, antioxidant and cytotoxicity studies.Macroscopic and microscopic description of medicinal plant is the first step. towards establishing the identity of the plant.The stock solution of the extraction was subjected to various preliminary phytochemical tests for qualitative determination of phytoconstituents present in plant. The study reveals that Agaricus Bisporus contains glycosides, carbohydrates, proteins, tannins, flavonoids and steroids.The extract of mushroom was found to have antibacterial, antioxidant and cytotoxic properties.The result of the study showed that the ethanolic extract of the Agaricus Bisporus is having more inhibition activity against gram ve bacteria in comparison with gram+ve bacteria.

The study also revealed that the extract is having antioxidant activity, but less effective then standard vitamin C.And the results of BSL assay revealed that the extract was almost 100% lethal to brine shrimp at the concentration of 1000 µg/ml.Thus it can be concluded that mushroom coffee prepared with Agaricus Bisporus species could have the above health benefits that is antibacterial, antioxidant and cytotoxic activities.

BIBILIOGRAPHY-

1. Usman M, Murtaza G, Ditta A. Nutritional, medicinal, and cosmetic value of bioactive compounds in button mushroom (Agaricus bisporus): a review. Applied Sciences. 2021 Jun 26; 11(13):5943.

2. Rezaeian S, Pourianfar HR. Antimicrobial properties of the button mushroom, Agaricus bisporus: A mini-review. International Journal of Advanced Research. 2016; 4(1):426-9.

3. Atila F, Owaid MN, Shariati MA. The nutritional and medical benefits of Agaricus bisporus: a review. Journal of Microbiology, Biotechnology and Food Sciences. 2021 Jan 6:2021:281-6.

4. Shaik J. Study on preliminary phytochemical screening and anti ulcer activity of Agaricus bisporus. J Pharmacogn Phytochem [Internet]. 2018;7(5):2015-9.

5. Maratha mandal college

6. Ramachandran S, Vamsikrishna M, Gowthami KV, Heera B. Dhanaraju MD. Assessment of cytotoxic activity of Agave cantula using brine shrimp (Artemia salina) lethality bioassay. Asian J Sci Res. 2011; 4(1):90-4.

7. Lewan L, Andersson M, Morales-Gomez P. The use of Artemia salina in toxicity testing. Alternatives to Laboratory Animals. 1992 Apr; 20(2):297- 301.

8. Almeida AA, Farah A, Silva DA, Nunan EA, Glória MB. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. Journal of agricultural and food chemistry. 2006 Nov 15;54(23):8738-43.

9. Biradi M, Hullatti K. Screening of Indian medicinal plants for cytotoxic activity by brine shrimp lethality (BSL) assay and evaluation of their total phenolic content. Drug Dev Ther. 2014; 5:139-44.

10. Sharma MV, Sagar A, Joshi M. Study on antibacterial activity of Agaricus bisporus (Lang.) Imbach. International Journal of Current Microbiology and Applied Sciences. 2015;4(2):553-8.

11. Rezaeian S, Pourianfar HR. Antimicrobial properties of the button mushroom, Agaricus bisporus: A mini-review. International Journal of Advanced Research. 2016;4(1):426-9.

12. Elhusseiny SM, El-Mahdy TS, Awad MF, Elleboudy NS, Farag MM, Aboshanab KM, Yassien MA. Antiviral, cytotoxic, and antioxidant activities of three edible agaricomycetes mushrooms: Pleurotus columbinus, Pleurotus sajor-caju, and Agaricus bisporus. Journal of Fungi. 2021 Aug 8;7(8):645.

13. Sarah QS, Anny FC, Misbahuddin M. Brine shrimp lethality assay. Bangladesh J Pharmacol. 2017 Jun 5;12(2):186-9.

14. https://www.healthline.com/nutrition/mushroom-coffee

15. https://www.caffeineinformer.com/caffeine-content/mushroom-coffee

16. Mukherjee KP. Quality control of herbal drugs. New Business Horrizons

17. Joshi VK, Joshi A, Dhiman KS. The Ayurvedic Pharmacopoeia of India, development and perspectives. Journal of ethnopharmacology. 2017 Feb 2:197:32-8.

18. Bhushan A, Kulshreshtha M. The medicinal mushroom Agaricus bisporus: review of phytopharmacology and potential role in the treatment of various diseases. Journal of Nature and Science of Medicine. 2018 Jan 1;1(1):4.

19. Liu J, Jia L, Kan J, Jin CH. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (Agaricus bisporus). Food and chemical toxicology. 2013 Jan 1:51:310-6.

20. Roy DN, Azad AK, Sultana F, Anisuzzaman AS. In-vitro antimicrobial activity of ethyl acetate extract of two common edible mushrooms. The Journal of Phytopharmacology. 2016;5(2):79-82.

21. Morosanova MA, Fedorova TV, Polyakova AS, Morosanova El. Agaricus bisporus crude extract: characterization and analytical application. Molecules. 2020 Dec 18:25(24):5996.

22. Heleno SA, Diz P, Prieto MA, Barros L, Rodrigues A, Barreiro MF, Ferreira IC. Optimization of ultrasound-assisted extraction to obtain mycosterols from Agaricus bisporus L. by response surface methodology and comparison with conventional Soxhlet extraction. Food chemistry. 2016 Apr 15:197:1054-63.

23. Risan MH, Taemor SH, Muhsin AH, Hussan S. Antibacterial activity of Agaricus bisporus and Pleurotus ostreatus extracts against some gram negative and positive bacteria. Eur. J. Biomed. 2017; 4:9-15.

24. Waqas HM, Akbar M, Iqbal MS. Antibacterial and antioxidant activities of Agaricus bisporus (JE Lange) Imbach from Pakistan. Bangladesh Journal of Botany. 2019 Dec 31;48(4):1075-81.

25. Sarah QS, Anny FC, Misbahuddin M. Brine shrimp lethality assay. Bangladesh J Pharmacol. 2017 Jun 5:12(2):186-9.

26. Tasew T, Mekonnen Y, Gelana T, Redi-Abshiro M, Chandravanshi BS, Ele E, Mohammed AM, Mamo H. In vitro antibacterial and antioxidant activities of roasted and green coffee beans originating from different regions of Ethiopia. International Journal of Food Science. 2020 Aug 25:2020.

27. Daglia M, Papetti A, Grisoli P, Aceti C, Dacarro C, Gazzani G. Antibacterial activity of red and white wine against oral streptococci. Journal of Agricultural and Food Chemistry. 2007 Jun 27; 55(13):5038- 42.

28. del Castillo MD, Ames JM, Gordon MH. Effect of roasting on the antioxidant activity of coffee brews. Journal of agricultural and food chemistry. 2002 Jun 19:50(13):3698-703.

29. https://en.m.wikipedia.org/wiki/Agaricus_bisporus