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Research paper

Extraction and Chemo profiling of *Agaricus bisporus* at Vindhya Region.

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Abstract: *Agaricus bisporus* White button mushrooms are accounting for 35-45 % of growth with total mushroom production all over the world. This mushroom has variable antimicrobial and medicinal application. Extraction of *Agaricus bisporus* grown and cultivated at Laboratory scale in the Vindhya region was performed to study the Chemo profiling with High Performance Liquid Chromatography. The solvents selected for extraction were pure distilled water 80% ethanol, and Ethyl acetate. The percentage of quercetin in ethanolic extract was reported to be 2.11% while the percentage of quercetin in ethyl acetate extract was reported to be 0.487% during High Performance Liquid Chromatography analysis. The results given in this report are only for the purpose of academic research and must not be understood or used as diagnostic report.

Keywords: *Agaricus bisporus,* Cultivation, Extraction, Chemo Profiling, HPLC

Abbreviations: High Performance Liquid Chromatography (HPLC)

INTRODUCTION:

Agaricus bisporus comes under the category of a food which is beneficial for humane health with excellent levels of dietary fibers and antioxidants including vitamins namely; thiamine, ascorbic acid, vitamin D_2 etc as well as minerals like folates, ergothioneine (ET) and polyphenols which may provide favorable effects on cardiovascular diseases and diabetes suggests that the mushroom might have potential anti-inflammatory, hypoglycemic, and hypocholesterolemic effects (Reed *et. al.*, 1995, Reed *et. al.*, 2001, Fukushima et. al. 2000, Koyyalamudi et. al., 2009). Around half of the fungal cell wall mass is constituted by β-glucans along with ergosterol, tocopherols, linoleic acid, and lectins. Fungus contains 1-6 mg of phenolics/g of dried mushroom and flavonoid concentrations ranged between 0.9 and 3.0 mg/g of dried matter; as myricetin and catechin (Koyyalamudi *et. al.*, 2009). Agaritine and its derivatives which chemically belongs to hydrazines, are the main aromatic compound of mushrooms. Hydrazines are present in mushroom species like *A. bisporus*, etc., Agaritine was found to contribute to the formation of toxic aryl

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diazonium ions. Gamma-glutaminy l-4-hydroxybenzene is the principal phenolic compound present in mushrooms (Mattila *et. al*., 2001, Valverde *et. al*., 2015). Abou-Heilah *et al*., (1987) reported potassium and sodium concentration was 300 and 28.2 parts per million, respectively (Beaulieu *et. al*., 1999). The ash analysis revealed the high amount of K, P, Cu, and Fe. (Espin *et. al*., 1999). *A. bisporus* contains Ca (0.04 g), Mg (0.16 g), *P* (0.75 g), Fe (7.8 g), Cu (9.4 mg), Mn (0.833 mg) and Zn (8.6 mg) per kilogram fresh weight. Various constituents, microelements, and vitamins

METHODOLOGY:

The methodology involved the collection of button mushrooms, their washing and cleaning, cutting into small pieces, shade drying at room temperature and pulverization into fine powder followed by defatting with petroleum ether. The solvents selected for extraction were pure distilled water 80% ethanol, and Ethyl acetate. The process of extraction (non-sequential) was done by Soxhlation, followed by concentration in water bath. The extraction substances were subjected to percentage yield analysis, organoleptic analysis and preliminary chemical analysis. Thin Layer Chromatography analysis of Extracts of qualitative detection of Quercetin equivalent flavonoidal compound, Quantitative HPLC analysis was done for Quercetin equivalent flavonoidal compound in the mushroom extracts.

Reagents and Chemicals

Quercetin was kindly provided by HiMedia. Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

Instrumentation

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ max. The HPLC system (Waters India Pvt Ltd) consisted of a pump, a U.V. Visible detector, a Thermo C18 (250 X 4.6 mm, 5µm) column, a Data Ace software.

Chromatographic Conditions

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml per minute. Sample volume of 20 µl used for each sample run, being injected into HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Preparation of standard stock solution

10 mg of Quercetin was weighed accurately and transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000 ppm.

Preparation of working standard solution

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. From this solution 1 ml solutions were transferred to 10 ml volumetric flasks followed by suitable dilution to prepare standard solution of Quercetin with 5, 10, 15, 20, 25 µg/ml concentration.

Analysis of Sample

Sample Preparation: 10 mg sample taken in 10 ml volumetric flask and dilute up to the mark with Methanol; resultant solution changed into filtered via Whatmann filter paper, sooner or later volume made as much as mark with equal solvent to obtain concentration of 1000 µg/ml.

RESULT AND DISCUSSION:

The observed Results are attached in the form of 3 separate sections ahead in this Study that includes the Results of Phytochemicals extraction and preliminary

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analysis; Results of TLC for qualitative detection of marker flavonoid; and Results of quantitative HPLC analysis of marker flavonoid in samples.

Table 1: Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Acetonitrile	50
Methanol	50
Flow rate	1ml/min
Temperature	Ambient Temp.
Sample Size	20 □l
Detection wavelength	256 nm
Retention time	2.50± 0.5 min

Identification of marker compound (Quercetin) by HPLC

A reverse phase C-18 column equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. Mobile phase was filtered through Whatmann filter paper and degassed. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 256 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The sample solution was chromatographed and a concentration of quercetin in Extract sample was found out using regression equation.

Table 2: Standard concentration of quercetin and generated area under peak

S.N.	Concentration in µg/ml	Area under peak
1	5	110.256
2	10	225.658
3	15	313.247
4	20	430.159
5	25	512.369

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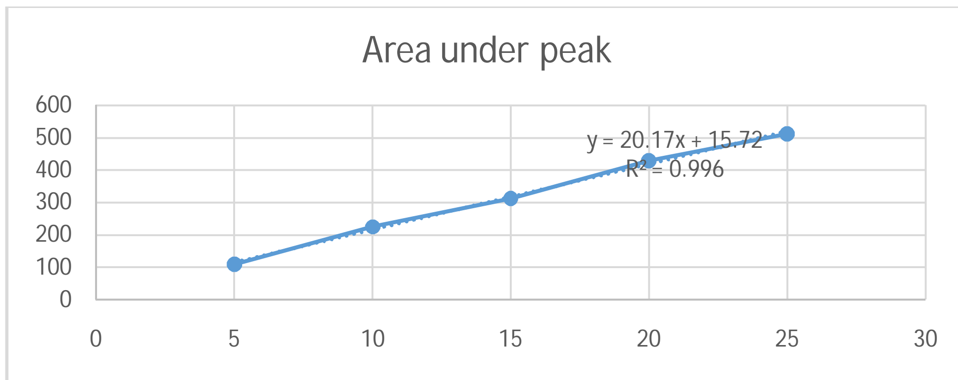

Figure 1: Standard concentration of Quercetin and Generated area under peak

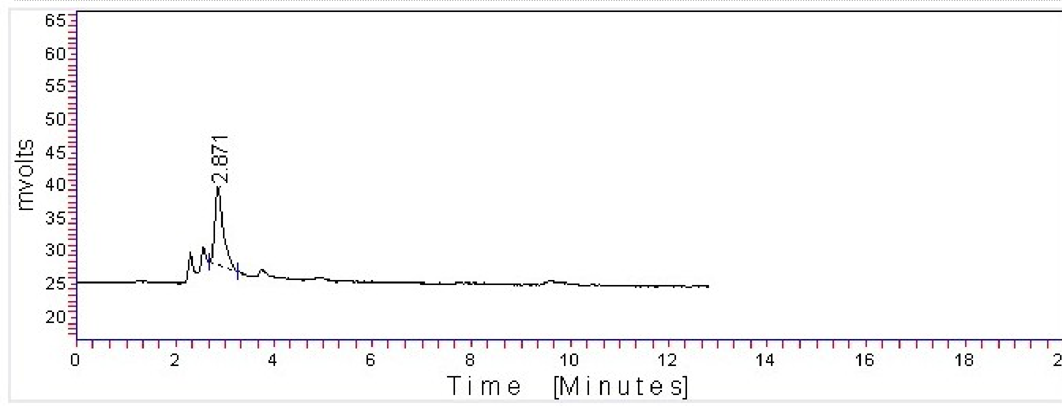

Figure 2: Chromatogram of Standard Quercetin

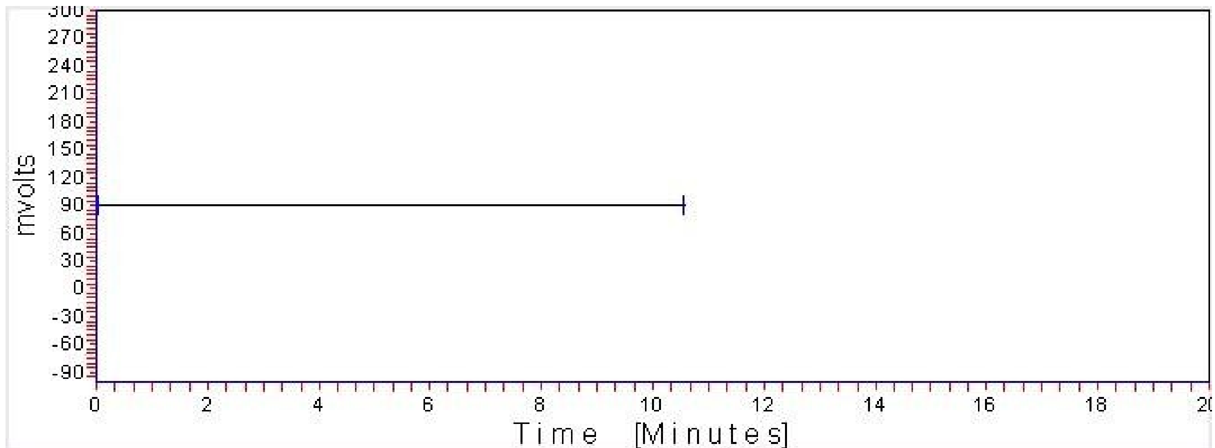

Figure 3: Chromatogram of Aqueous extract of *A. bisporus* for detection of quercetin marker

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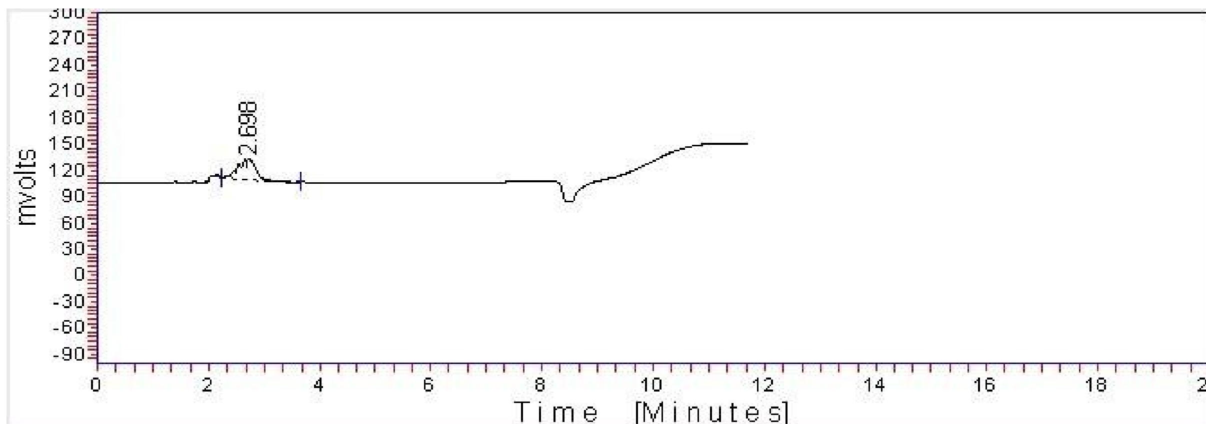

Figure 4: Chromatogram of Ethanolic extract of *A. bisporus* for detection of quercetin marker

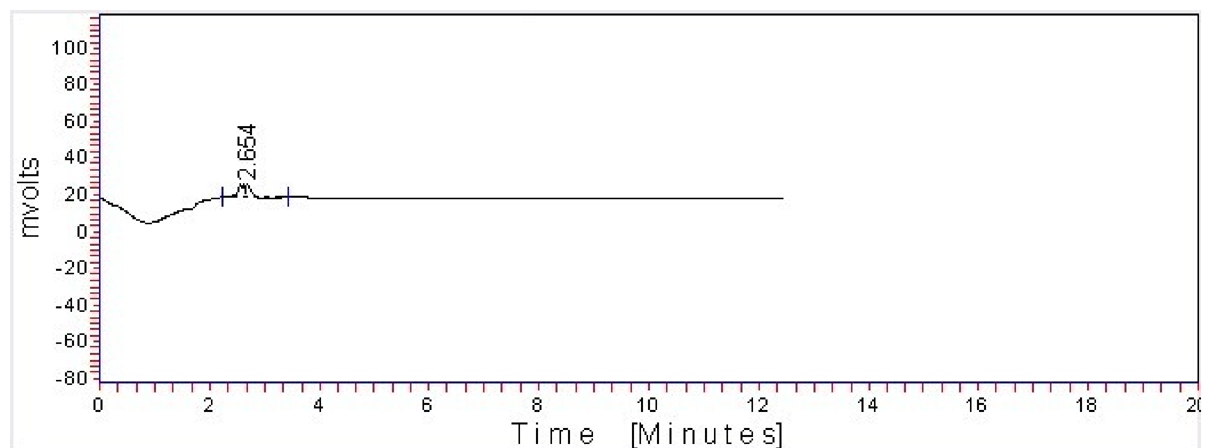

Figure 5: Chromatogram of Ethyl acetate extract for detection of quercetin marker

Detected percentage of Marker flavonoid Quercetin in samples

Standard Plot for know concentration of *Quercetin Standard* with respect to peak values in the form of mvolts in particular chromatographic conditions gives the area under peak in order to compare the percentages concentration of marker compound in test sample extracts. The Graph is obtained from Excel 2010 linear regression function (figure 1). The percentage of marker compound quercetin in the test samples extracts of mushroom extracts are depicted in table 3 of this section C of report – I.

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Table 3: The peak values obtained in HPLC chromatogram in the form of mvolts for button mushroom extracts comparable with standard to describe the percentage of the marker flavonoid compound quercetin in the extracts.

S.N.	Extracts	Concentration (µg/ml)	Peak Values (in mvolts)	Percentage Quercetin
1	*Aqueous Extract*	1000 µg/ml.	000	Nil
2	*Ethanolic Extract*	1000 µg/ml.	2.698	2.110%
3	*Ethyl Acetate Extract*	1000 µg/ml.	2.654	0.497%

CONCLUSION:

It is observed that, out of the 3 extracts prepared from mushrooms (*A. bisporus*) namely aqueous extract, ethanolic extract and ethyl acetate extract, only ethanolic and ethyl acetate extract responded for detection of quercetin like flavonoid in samples during HPLCanalysis. Although, flavonoind were reported present in all the 3 extracts tested during preliminary chemoprofiling of button mushroom as depicted in section A of this report, but the specific marker flavonoid compound quercetin was reported nil in HPLC analysis. The percentage of quercetin in ethanolic extract was reported to be 2.11% while the percentage of quercetin in ethyl acetate extract was reported to be 0.487% during HPLC analysis. The results given in this report are only for the purpose of academic research and must not be understood or used as diagnostic report. Also the results may vary depending on the methods, techniques of experiments by individual and laboratory/place of experimentation. Reed et. al., 1995, Reed et.al., 2001, Fukushima et. al. 2000, Koyyalamudi et. al., 2009 Koyyalamudi et. al., 2009 Mattila et.al., 2001, Valverde et.al., 2015

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