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Efficacy of selected botanicals on biochemical constituents of white button mushroom [*Agaricus bisporus* (Lange) Imbach]

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Abstract
The study was carried out to determine efficacy of selected botanicals *viz., Azadirachta indica, Lantana camara* and *Allium cepa,* on protein, carbohydrate and lipid contents of white button mushroom [*Agaricus bisporus* (Lange) Imbach]. Protein content ranged from 26.27 to 29.58 g/100g, carbohydrate content ranged from 17.10 to 20.57 g/100g and total lipid content ranged from 2.20 to 3.02 g/100g (on dry weight basis). A qualitative phyto-chemical analysis was also performed for the detection of secondary plant metabolites. Fresh leaves of selected botanicals were collected, dried and extracted using 95% ethanol. Phytochemical analysis gave positive results for saponins, tannins, glycosides, reducing sugars, alkaloids, flavonoids, volatile oils and terpenoids.

Keywords: *Agaricus bisporus,* biochemical analysis, botanicals, phyto-chemical analysis

Introduction
The use of mushrooms as food is probably as old as civilisation and mushrooms currently have greater importance in the diet of mankind. Cultivation and production of edible mushrooms are on the increase, particularly in Europe, America and Asia. The increased nutritional importance is due to the nutritive value of high-grade mushrooms, which almost equals that of milk (Shu *et al.,* 2007) [31]. Mushrooms have been evaluated for their nutritional status on the basis of their chemical composition. Cultivated and wild mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, fibres and vitamins (Aida *et al.,* 2009 and AOAC, 1995) [1, 3]. Barros *et al.* (2008) [5] reported that the wild mushrooms were richer sources of protein and had a lower amount of fat than commercial mushrooms. Wild mushroom proteins also contain considerable amounts of non-essential amino acids such as: alanine, arginine, glycine, glutamic acid, aspartic acid, proline and serine. They are important in providing structure to cells, tissues and organs and therefore essential for growth and repair (Beluhanm and Ranogajec, 2011) [6].
Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983) [4]. Due to high amount of proteins they can be used to bridge the protein malnutrition gap. The mushrooms are used as functional foods as they are used as nutrient supplements and to enhance immunity in the form of tablets. Overall, the world production of mushrooms is dominated by those mushrooms which are both edible and have medicinal properties.
The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered (Raja *et al.,* 2009) [28]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found In-vitro to have medicinal properties.
Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswas *et al.,* 2002) [8]. Phyto-chemicals from medicinal plants serve as lead compounds in antimicrobial discovery (Chakravarthy and Gode, 1985; Ebi and Ofoefule, 2000; and Cohen, 2002) [12, 16, 13]. The aim of this investigation was to analyze the nutritional values of white button mushroom, with a goal of increasing awareness of the beneficial effects of edible mushrooms among the consumers.

Materials and Methods

The experiment was conducted at Mushroom Crop Room, Department of Plant Pathology, SHUATS, Allahabad. Cultivation of white button mushroom (*Agaricus* bisporus) was carried out in two consecutive years i.e., 2014-15 and 2015-16. The cultivation technique of white button mushroom is divided into four major steps *viz*., composting, spawning, casing and harvesting. The spawn of *A. bisporus* was procured from Directorate of Mushroom Research, Solan (HP) and the strain of *A. bisporus* spawn was DMR-3. Wheat straw was used as substrate for cultivation of white button mushroom (*A. bisporus*). Compost was made using long method (28 days).

The dried powder of selected plant materials (*Azadirachta indica, Lantana camara* and *Allium cepa*) was incorporated separately in the compost @ 1, 2 and 3% (w/w) and filled in polythene bags @ 500 g of compost. The untreated bags (devoid of botanicals) were kept as control. All the treatments including control were replicated six times. Spawn of *A. bisporus* was added @ 7.5g/kg of compost (Kapoor, 2004). Then, the bags were incubated inside the Mushroom Crop Room, where temperature (20±20C) and humidity (80-85%) was maintained. Room having spawn running bags was kept in dark for 10-15 days till complete colonization of the compost with fungal mycelium (El-Kattan and El-Hadded, 1998) [17].

Estimation of Protein

The protein analysis of the white button mushroom has been done by using Lowry's method.

Estimation of carbohydrate

The carbohydrate analysis of the white button mushroom has been done by using the anthrone sulfuric acid method given by Hedge and Hofreiter (1962) [20].

Estimation of lipid

Total lipid was determined by slight modified method of Folch *et al*. (1957) [18].

Collection of botanicals

Fresh, healthy and uninfected leaves of *Azadirachta indica, Lantana camara* and *Allium cepa* were collected from campus of SHUATS, Allahabad. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly and dried.

Preparation of Extracts

The botanicals *viz., Azadirachta indica* & *Lantana camara* (leaves) and *Allium cepa* were washed with distilled water and oven dried at a temperature of 80 °C for 24 hours, grounded into fine powder and extracted separately using 100 ml of 95% concentration of ethanol and distilled water and filtration was done with Whatman Filter Paper (No. 1).

Phytochemical analysis

To test for the presence of the saponins, tannins, reducing sugars, glycosides, alkaloids, flavonoids, volatile oils, and terpenoids, following methods were used:

a) Saponins

Saponins were detected using the froth test. 1ml of the sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

b) Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for gallic tannins and green color indicates for catecholic tannins.

c) Reducing Sugars

To 0.5ml of plant extracts, 1 ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

d) Glycosides

Twenty five ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

e) Alkaloids

Two ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

f) Flavonoids

Four ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

g) Volatile oils

Two ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl was added. A white precipitate is formed if volatile oils are present.

h) Terpenoids

Four ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids. (Talukdar and Choudhary, 2010) [32].

Results and Discussion

It is evident from the table 1 and 2 that in both the years (2014-15 and 2015-16), there were significant difference between the influences of plant extracts on protein content of *A. bisporus*. Maximum protein content (g/100g of mushroom) was recorded with treatment T_3 (*A. cepa*) (28.26 g and 28.40 g) followed by T_1 (*A. indica*) (27.77 g and 27.92 g) and T_2 (*L. camara*) (27.58 g and 27.84 g) as compared to control (26.68 g and 26.77 g) in both the years (2014-15 and 2015-16), respectively.

It is also evident from the table 3 and 4 that in both the years (2014-15 and 2015-16), there were significant difference between the influences of plant extracts on carbohydrate content of *A. bisporus*. Maximum carbohydrate content (g/100g of mushroom) was recorded with treatment T_3 (*A. cepa*) (19.21 g and 19.39 g) followed by T_1 (*A. indica*) (18.71 g and 18.79 g) and T_2 (*L. camara*) (18.58 g and 18.75 g) as compared to control (17.88 g and 18.03 g) in both the years (2014-15 and 2015-16), respectively.

Data presented in the table 5 and 6 also revealed that in both

the years (2014-15 and 2015-16), there were non significant difference between the influences of plant extracts on lipid content of *A. bisporus*. Maximum lipid content (g/100g of mushroom) was recorded with treatment T_1 (*A. indica*) (2.67 g and 2.72 g) followed by T_3 (*A. cepa*) (2.66 g) and T_2 (*L. camara*) (2.62 g and 2.66 g) as compared to control (2.5 g and 2.42 g) in both the years (2014-15 and 2015-16), respectively.

It was further observed that protein content increased on increasing the concentrations (1, 2 and 3%) of plant extracts in both the years (2014-15 and 2015-16).

These results were in accordance with the result of Colak *et al*. (2007) [14], who recoded protein (19.13-23.06%) and carbohydrate (3.05-5.38%) of *A. bisporus* using different casing soils. Coskuner and Ozdemir (1997) [15] reported that the protein and carbohydrates levels of mushroom samples are within the range of 19-35% and 4-8.1% respectively, of the dry weight basis. Boda *et al*. (2012) [11] recorded protein content (2.20g), carbohydrate content (4.85g) and lipid content (2.38g) of *A. bisporus.*

The results of qualitative phyto-chemical compounds of ethanol and water extracts neem leaves showed the presence of saponins, tannins, glycosides, volatile oils, reducing sugars, alkaloids, flavonoids and terpenoids (Table 7).

This class of compounds independently or in combination may be the probable reason for the present findings. *Azadirachta indica* (Neem) contains at least 35 biologically active principals of which triterpenoides, nimbin and azadirachtin present predominantly in the seeds, leaves and other parts of the neem (Mondall *et al*., 2009; Nahak and Sahu, 2010) [23, 24].

Biu *et al.* (2009) [9] observed the presence of saponins, tannins, glycosides, alkaloids, terpenes and flavenoids in leaf extracts of neem. Bennett and Wallsgrove (1994) [7] and Grayer and Harbourne (1994) observed that glycosides and saponins have antifungal activity. Osbourn (1996) [26] observed that many saponins exhibit potent antifungal activity and as a result have been implicated as determinants of a plant's resistance to fungal attack.

Table 1: Quantitative estimation of protein of *Agaricus bisporus* (dry weight basis) (2014-15)

Symbols	Treatments/ Botanicals	*Protein content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	26.27	27.52	29.52	27.77
T_2	*Lantana camara*	26.92	27.17	28.65	27.58
T_3	*Allium cepa*	27.43	28.27	29.08	28.26
CD @0.05 (Treatment)					0.454
CD @0.05 (Concentration)					0.454
CD @0.05 (Treatment × Concentration)					0.786
S.E. (d)					0.387

Control (T_0) – 26.68 g, *Mean of six replications;

Table 2: Quantitative estimation of protein of *Agaricus bisporus* (dry weight basis) (2015-16)

Symbols	Treatments/ Botanicals	*Protein content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	26.43	27.73	29.58	27.92
T_2	*Lantana camara*	27.15	27.68	27.68	27.84
T_3	*Allium cepa*	27.45	28.47	29.28	28.40
CD @0.05 (Treatment)					0.336
CD @0.05 (Concentration)					0.336
CD @0.05 (Treatment × Concentration)					0.583
S.E. (d)					0.287

Control (T_0) – 26.77 g, *Mean of six replications

This result was in accordance with the result of Blumenthal (1976). Raya *et al.* (2014) recorded protein content (33.85%) and carbohydrate content (42.56%) of *A. bisporus.* Similar results were recorded by Teklit (2015) [33], who recorded protein content (41.06%) and carbohydrate content (28.38%). Similar findings have also been reported by Alam *et al.* (2008) [2]; Parashare *et al.* (2013) [27]; Nasiri *et al*. (2013) [25] and Rana *et al.* (2015) [29].

Table 3: Quantitative estimation of carbohydrate of *Agaricus bisporus* (dry weight basis) (2014-15)

Symbols	Treatments/ Botanicals	*Carbohydrate content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	17.10	18.52	20.52	18.71
T_2	*Lantana camara*	17.92	18.18	19.65	18.58
T_3	*Allium cepa*	18.43	19.13	20.07	19.21
CD @0.05 (Treatment)					0.478
CD @0.05 (Concentration)					0.478
CD @0.05 (Treatment × Concentration)					0.828
S.E. (d)					0.408

Control (T_0) – 17.88 g, *Mean of six replications

Table 4: Quantitative estimation of carbohydrate of *Agaricus bisporus* (dry weight basis) (2015-16)

Symbols	Treatments/ Botanicals	*Carbohydrate content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	17.20	18.62	20.57	18.79
T_2	*Lantana camara*	18.08	18.35	19.82	18.75
T_3	*Allium cepa*	18.65	19.32	20.22	19.39
CD @0.05 (Treatment)					0.434
CD @0.05 (Concentration)					0.434
CD @0.05 (Treatment × Concentration)					0.752
S.E. (d)					0.371

Control (T_0) – 18.03 g, *Mean of six replications

Table 5: Quantitative estimation of lipid of *Agaricus bisporus* (dry weight basis) (2014-15)

Symbols	Treatments/ Botanicals	*Lipid content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	2.30	2.72	3.00	2.67
T_2	*Lantana camara*	2.20	2.72	2.93	2.62
T_3	*Allium cepa*	2.22	2.80	2.97	2.66
CD @0.05 (Treatment)					NS
CD @0.05 (Concentration)					0.181
CD @0.05 (Treatment × Concentration)					NS
S.E. (d)					0.154

Control (T_0) – 2.5 g, *Mean of six replications

Table 6: Quantitative estimation of lipid of *Agaricus bisporus* (dry weight basis) (2015-16)

Symbols	Treatments/ Botanicals	*Lipid content (g/100g)			
		Concentration (%)			
		1	2	3	Mean
T_1	*Azadirachta indica*	2.40	2.73	3.02	2.72
T_2	*Lantana camara*	2.25	2.80	2.92	2.66
T_3	*Allium cepa*	2.27	2.75	2.95	2.66
CD @0.05 (Treatment)					NS
CD @0.05 (Concentration)					0.176
CD @0.05 (Treatment × Concentration)					NS
S.E. (d)					0.150

Control (T_0) – 2.42 g, *Mean of six replications

Phyto-chemical analysis

The results of qualitative phyto-chemical compounds of selected plant extracts are given in table 7.

Table 7: Phyto-chemical components of selected plant extracts

Sr. No.	Chemical constituent	*Azadirachta indica*		*Lantana camara*		*Allium cepa*	
		Ethanol	Water	Ethanol	Water	Ethanol	Water
1.	Saponins	+	+	+	+	-	+
2.	Tannins	+	+	+	+	+	+
3.	Glycosides	+	-	-	+	-	+
4.	Reducing Sugars	+	+	+	+	+	+
5.	Alkaloids	+	+	+	+	-	+
6.	Flavonoids	+	+	-	+	+	+
7.	Volatile oils	+	-	+	-	+	-
8.	Terpenoids	+	+	+	-	-	-

(+) indicates present and (-) indicates absent

In conclusion, white button mushroom contains high protein content with moderate carbohydrate and lipid contents. Overall, the rich nutritional composition makes cultivated mushrooms very special. So, mushrooms are a promising food that may overcome protein-energy malnutrition problem in the third world. The protein, carbohydrates and lipid content make them ideal vegetable for diabetic, cancer and heart patients. These nutrients contents made mushroom as a low energy, healthy foodstuff and these mushrooms may also be used as protein supplementary diet.

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