

Phytochemical Qualitative Analysis and Immunomodulator Activity of *Agaricus bisporous* Ethanol Extract by Carbon Clearance Technique

Ashok A¹ and Shabudeen P.S.^{2*}

¹Department of Chemistry, Kumaraguru College of Technology, Coimbatore, India

²Approved Research Centre for Chemistry and Environmental Science, Principal Project Investigator, CO₂ Sequestration Research, DST, Govt of India, Kumaraguru College of Technology, Coimbatore, India

Abstract

The *Agaricus bisporous* have been collected and its extracts are subjected to qualitative analysis and immunomodulator activity. Quantitative analysis has been carried out to find some phytochemicals presents in the sample. These Ethanol extracts revealed with the presence of the constituents such as Sterols, Saponins, Coumarin Glycosides, Amino acid/protein, Resins. A preliminary phytochemical screening has been conducted with the selected fungal extracts by using standard C.K.Kokate procedures and it is revealed that several secondary metabolites are present in the sample. *In vivo* the effect of ethanol extract of the two *Agaricus bisporous* has been applied on neutrophil phagocytosis activity. Various concentrations namely 50, 100 and 200 µg/ml of the ethanol extract has been tested for phagocytosis as neutrophil locomotion. This has been tested with Swiss albino mice with selected dose of ethanol concentrations in 50, 100 and 200 mg/kg body weight has been administrated in order to confirm this with macrophage phagocytosis activity by carbon clearance test. *In vivo* studies of *Agaricus bisporous* reveals the moderate immune stimulator activity, thus this fungal species have demonstrated the prominent immunostimulator activity. The data generated in this study has the basics for its use as the therapeutic both as traditional and folk medicine as a widely as to be practiced.

Keywords: *Agaricus bisporous*; Phagocytic index; Ethyl acetate fraction; 1.0% Sodium carboxy methyl cellulose; Carbon clearance test

Introduction

The mushrooms are largely used as a foodstuff from historical details as a traditional food and it have greater importance in the diet of mankind most recently. The cultivation and production of edible mushrooms are on the increase, particularly in Europe, America and Asia due to its increased nutritional values. Their increased nutritional importance is almost equals to that of the milk [1]. The cultivated and wild mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, fibers and vitamins [2-5]. The available literature review indicates that mushrooms have phytochemicals with the compounds of strong antioxidants [6]. The phenolic compounds, alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides have been detected and reported with wild mushrooms of Sudan's and Nigerian regions of African continent [7]. Immune modulation is a bio-chemical phenomenon which is explained "as any change in the immune response and may involve induction, expression, amplification of any part or phase in the immune response of the organism". The effect of modulation may be very specific and limited to a given antigen of an agent or a non-specific subtract, with a great effect on immune responsiveness. The stimulation of the immune response is preferred with certain type of people namely immune compromised patient. Whereas, it suppresses the immune responsiveness of transplant recipient or patient who are auto allergic or prone with inflammatory diseases [8]. Several *in vivo* and *in vitro* models are available for screening immuno modulation activity [9] in which Phagocytosis is one of the extensively adopted methods for screening the immune response.

Materials

Collection of sample

The mushrooms have been collected from the neighboring places of Coimbatore City. This shade dried mushrooms are used for this present investigation with standard procedures and protocols.

Extract preparation by cold maturation method: The accurately

weight 150 g of power sample has been collected and it is mixed with 100 ml of 50% ethanol in a round bottom flask and kept for incubation for 24 hrs. Then this mixture has been filtered and the filtered sample is kept in the evaporation stand at 70°C. The dried sample is collected and allowed for the phytochemical screening and Immunomodulatory activities.

Animals used: Swiss albino mice of either sex of Indian region, weighing 18-25 g each, have been selected for this study with proper permissions. They are housed under standard conditions at the temperature of 25 ± 10°C and at a relative humidity of 60 ± 10%, for 12/12 h light/dark cycle, and they are fed with standard pellets and water. These Animal experiments were performed in accordance with the CPCSEA norms and are obtained from Nandha College of Pharmacy, Animals House.

Methods

The qualitative phytochemical analysis

The phytochemical analysis carried out with the standard procedure, adopted by Kokate, in which, 1 g the sample is transferred into a clean screw cap bottle of 10 ml capacity and a solvent ethanol, 10 ml is added into it separately and the extracts are separated and collected and stored at 4°C which are used for the remaining analysis. The tests for Sterols,

***Corresponding author:** Shabudeen P.S.S, Approved Research Centre for Chemistry and Environmental Science, Principal Project Investigator, CO₂ Sequestration Research, DST, Govt of India, Kumaraguru College of Technology, Coimbatore-641049, India, Tel: 9597956097; E-mail: ashokbiotech16@gmail.com

Received: January 29, 2015; **Accepted:** March 17, 2015; **Published:** March 24, 2015

Citation: Ashok A, Shabudeen PSS (2015) Phytochemical Qualitative Analysis and Immunomodulator Activity of *Agaricus bisporous* Ethanol Extract by Carbon Clearance Technique. Biochem Pharmacol (Los Angel) 4: 168. doi:10.4172/2167-0501.1000168

Copyright: © 2015 Ashok A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Saponins, Flavonoids, Coumarin glycosides, Anthraquinone glycosides, Amino acid/Protein, Lactone, Resins, Alkaloids, Tri-terpenes, Carbohydrates, Cynophoric glycoside, Tannins are all performed and is tabulated.

Systematic and qualitative studies are carried out as per the prescribed by standard protocols with the crude drug samples on primary and secondary metabolites. The extracts of the algae species are subjected to standard chemical tests as a normal routine procedure in order to identify the phyto constituents followed by the protocol adopted by Harborne.

In-vivo immunomodulation study

Carbon clearance test: Swiss albino mice were divided into 4 groups, each containing 6 animals. Group I (control) was given 1.0% sodium carboxy methyl cellulose (CMC) in water (0.3 ml/mouse) for 5 days. Group II-IV were given different concentrations of ETF (50, 100 and 200 mg/kg, p.o.) for 7 days. At the end of 7th day, after 48 hrs, mice were injected via the tail vein with carbon ink suspension (1:50 dilution of Indian ink, Camel, and 10 µl/gm body wt.). Blood samples were withdrawn (in EDTA solution 5 µl) from the retro-orbital vein at 0 and 15 min, a 25 µl sample has been mixed with 0.1% sodium carbonate solution (2 ml) and its absorbance at 650 nm has been determined. The rate of carbon clearance (phagocytic index k) is calculated from the slope of each time-concentration curve drawn by plotting as ordinate on semi logarithmic paper against time as abscissa. Results are expressed and keeping with the arithmetic mean ± S.E.M. of six mice [8,9].

Statistical Analysis

All experiments have been repeated thrice and results are expressed as mean ± SD.

Results

The phytochemical screenings of the *Agaricus bisporus* fraction are performed. Sterols, Saponins, Coumarin glycosides, Amino acid/protein, Resins showed the positive results for ethanol extract; while Alkaloids, Tri-terpenes, Carbohydrates, Flavonoids, Anthraquinone glycosides, Cynophoric glycoside, Lactone, Tannins showed the negative results (Table 1). It has been revealed that *Agaricus bisporus* has the stimulated chemotatic, phagocytic and intracellular killing of human neutrophils at a dose of 100 µg/ml and 200 µg/ml respectively. *In vivo* studies of *Agaricus bisporus* have shown moderate immunostimulator activity at a concentration of 200 mg/ml as compared with control, whereas fungal species have shown prominent immunostimulator activity at the same dose (Table 2).

S. No	Test	Ethanol extract
1	Sterols	Positive
5	Saponins	Positive
7	Coumarin glycosides	Positive
12	Amino acid/protein	Positive
13	Resins	Positive

Table 1: Qualitative Phytochemical screening of *Agaricus bisporus*.

Time in minutes	Animal groups (Values are in mean ± SEM)			
	Control	50mg/kg	100mg/kg	200mg/kg
0	0.019 ± 0.001	0.020 ± 0.000	0.022 ± 0.000	0.024 ± 0.000
15	0.006 ± 0.001	0.008 ± 0.001	0.011 ± 0.000	0.015 ± 0.001

Table 2: Effect on *Agaricus bisporus* on Carbon clearance test.

Discussion

The use of immunostimulants, mostly as adjuvant to chemotherapy, to control and prevention of infection holds great promise [10-12]. Significant interest has now been generated in research on bioactive molecules from *Agaricus bisporus* designated as immunomodulatory agents in alternate systems of medicine. The ethanol fraction of *Agaricus bisporus* was tested in the present study to determine their possible effect on immune function at three dose levels. This investigation reveals the ethanol fractions have significantly increased the phagocytic function of human neutrophils, when compared with control and increase the movement of neutrophils towards the foreign body which is the most important step in the phagocytosis process or activity.

The ethanol extracts of *Agaricus bisporus* on *in vivo* carbon clearance test have shown moderate immunostimulatory effect at a dose of 100 mg/kg body weight po route. This may suggest that macrophage probably stimulate production of cytokines which in turn stimulates other immunocytes which may help in defence mechanisms of body to counter various infections. A preliminary observation of these *Agaricus bisporus* extracts also requires further investigations for exact mechanism of immunomodulation.

Acknowledgement

The authors are thankful to Dr. R. Duraisamy, Associate Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamilnadu, India who provides the animal house facilities.

References

- Chang ST, Miles PG (1989) In Edible Mushrooms and Their Cultivation. CRC Press, Boca Raton, USA 293-302.
- Alofe FV (1991) Amino acids and trace minerals of three edible wild mushrooms from Nigeria. J Food Compost Anal 4: 147-174.
- Aletor VA (1995) Compositional studies on edible tropical species of mushrooms. Food Chem 54: 265-268.
- Oei P (1996) Mushroom Cultivation with Special Emphasis on Appropriate Techniques for Developing Countries. Tool Publications, Leiden, Netherlands, pp. 3-5.
- Stamets P (2000) Growing Gourmet and Medicinal Mushrooms. Ten Speed Press, California, USA, pp. 207-430.
- Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18: 872-879.
- Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO (2012) Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius*. J Microbiol Biotech Res 2: 366-374.
- Sell S (1987) Immunology Immunopathology and Immunity. Elsevier Science Publishing Co Inc, New York, USA, pp. 655-683.
- Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA (1990) Immunomodulatory activity of methanolic extracts of *Pongamia glabra* Vent seeds and bark in cyclophosphamide induced mice. Indian Drugs 28: 56-63.
- Jayathirtha MG, Mishra SH (2004) Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica*. Phytomedicine 11: 361-365.
- Biozzi G, Benacerraf B, Halpern B (1953) Quantitative study of the granuloplectic activity of the reticuloendothelial system. Br J Exp Pathol 34: 426-440.
- Chatterjee RK, Fatma N, Jain RK, Gupta CM, Anand N (1988) *Litomosoides carinii* in rodents: immunomodulation in potentiating action of diethylcarbamazine. Jpn J Exp Med 58: 243-248.