International Journal of Microbiological Research 1 (3): 110-113, 2010
ISSN 2079-2093

Proximate Analysis and Antibacterial Activity of an Edible Mushroom Volvariella Bombycina

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Abstract: Mushroom has been valued throughout the world as both food and medicine for thousands of years. In the present study proximate analysis and antibacterial activity of an edible mushroom *Volvariella bombycina* was evaluated. Proximate analysis includes total dietary fiber, crude protein, lipid, ash, moisture and carbohydrate content in mycelia and fruiting body of *V. bombycina*. The antibacterial activity of *V. bombycina* extracts (Hexane, Chloroform, Methanol and Ethyl acetate) were evaluated against the clinically important bacterial strains *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775) *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 10145), by agar well diffusion method. Mycelia of *V. bombycina* showed 31.8 % dietary fiber, 25.5% crude protein, 1.15% lipid, 9.03% ash, 8.75% moisture and 34.75% carbohydrate content, while fruit body showed 24.6% dietary fiber, 28.3% crude protein, 2.72% lipid, 10.9% ash, 9.68% moisture and 38.9% carbohydrate content. Results from the proximate analysis of *V. bombycina revealed* that rich in protein with low lipid content make *V. bombycina* is an ideal food for a good health. The antibacterial activity of *V. bombycina* showed ethyl acetate extract have maximum inhibitory activity against the investigated bacterial strains followed by methanol, chloroform and hexane extracts. Hexane and chloroform extracts were totally inactive against *P. aeruginosa*. *B. subtilis* was the most susceptible Gram-positive bacteria followed by *Stap. aureus*. *P. aeruginosa* was the most resistant Gram-negative bacterial strain followed by *K. pneumonia* and *E. coli*. The results of the present study supported the usage of the studied mushroom as ideal food and suggested that *V. bombycina* extracts possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Key words: *Volvariella bombycina* · Antibacterial activity · Proximate analysis

INTRODUCTION

Mushrooms have long been valued as delicious and nutritional food in many countries. Mushrooms, are initially consumed for their flavour, are now consumed because of their rich nutritional value in terms of protein, minerals and vitamins content and also medicinal properties [1]. Mushroom cultivation is gaining popularity due to low cost technology and easy availability of various substrates for its cultivation [2]. In India, prevalence of varied, agro-climatic conditions and availability of vast quantities of lignocellulosic raw materials have stimulated the cultivation of mushroom.

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Volvariella bombycina is an important edible mushroom belong to the family Pluteaceae, commonly called as Silky rosegill, Silver-silk straw mushroom. V. bombycina are appreciated for their chemical and nutritional characteristics [1]. V. bombycina were reported to have good antioxidant, antitumor and hypercholesterolemic effects [3]. The present study was designed to investigate the proximate analysis and antibacterial activity of an edible mushroom *V. bombycina*. Proximate analysis includes total dietary fiber, crude protein, lipid, ash, moisture and carbohydrate content in mycelia and fruiting body of *V. bombycina*. The antibacterial activity of *V. bombycina* extracts (Hexane, Chloroform, Methanol and Ethyl acetate) was evaluated against the clinically important bacterial strains *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775) *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 10145), by agar well discussion method.

MATERIALS AND METHODS

Mushroom Collection and Cultivation: The fresh basidiomata was collected from the dead wood of *Ficus bengalensis* at the Indian Institute of Technology, Chennai, India, during rainy season (in the month of August). Based on the macro and microscopical characteristics, the mushroom strain was confirmed as an edible mushroom belongs to the genus *Volvariella bombycina*. The *V. bombycina* mycelium was produced by growing fresh basidiomata in Potato Dextrose Broth (PDB) at 22°C for 14 days at pH 6.8. Fruit body of *V. bombycina* was produced by inoculating spawn in paddy straw substrate at 22°C for 28 days.

Proximate Analysis: The mycelium and fruit body of *V. bombycina* were analyzed for food composition according to the Association of Official Analytical Chemists [4]. Proximate analysis includes the determination of total dietary fiber, crude protein, lipid, ash, moisture and carbohydrate content.

Extract Preparation for Antibacterial Activity: The fruit body of *V. bombycina* was air dried under shade and made into coarse powder. The powder was soaked in respective extracts (hexane, chloroform, methanol and ethyl acetate) for 72hrs at room temperature (by maceration method) and the resultant was filtered through whatmann filter paper (3 cycle) and evaporated for dryness at 60°C in vacuo.

Well Diffusion Method: Antibacterial activity of the *V. bombycina* extracts were tested using Well diffusion method [5]. The organisms were maintained on an agar slope at 4°C were sub-cultured for 24 hrs before use. Isolated colonies of the bacteria were placed into individual tubes containing 5 ml of sterile brain-heart infusion broth (BHIB) (Himedia) and incubated at 37°C, before adjusting the tubes with 0.5 McFarland Units using sterile BHIB. Turbidity was also verified using spectrophotometric comparison with a 0.5 McFarland standard. The dilutions were used within 15 min of preparation and gently vortexed prior to use. The standardized inoculums (1.5×10^6 Colony forming unit (cfu)/ml 0.5 McFarland standards) was introduced on the surface of sterile Mueller-Hinton agar (pH 7.2–7.4) plates using sterile cotton swabs (by streak plate method). The inoculations were done along three axes in a rolling motion to ensure uniform bacterial distribution and growth. Wells were made on the agar surface with 5mm cork borer. 10 mg of each extracts were dissolved in 1 ml of dimethyl sulfoxide (DMSO) and 50µl of each extracts were introduced into the well using sterile syringe. Controls wells were maintained by introducing respective extract solvents into the well. The plates were incubated at 37°C for 24 hrs. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone including the well (in mm). The experiment was carried in triplicates to get concordant reading.

RESULTS

Proximate analysis of *V. bombycina* (dry powder) found to vary in mycelia and fruiting body. Mycelia of *V. bombycina* showed 31.8 % dietary fiber, 25.5% crude protein, 1.15% lipid, 9.03% ash, 8.75% moisture and 34.75% carbohydrate content, while fruit body showed 24.6% dietary fiber, 28.3% crude protein, 2.72% lipid, 10.9% ash, 9.68% moisture and 38.9% carbohydrate content (Table 1).

All the crude extracts of *V. bombycina* showed antibacterial activity against the gram positive bacteria *B. subtilis* and *Stap. aureus*, but the activity of inhibition varied for the bacteria with respect to the type of extract (Table 2). Ethyl acetate extract showed maximum inhibitory activity against the investigated bacterial strains followed by methanol, chloroform and hexane extracts. Hexane and chloroform extracts were totally

Table 1: Proximate analysis of an edible mushroom *Volvariella bombycina*

S. No	Proximate analysis	Fruiting body (%)	Mycelia (%)
1	Crude fiber	24.60	31.80
2	Protein	28.30	25.50
3	Lipid	2.72	1.15
4	Total ash	10.90	9.03
5	Moisture content	9.68	8.75
6	Carbohydrate	38.90	34.75

Table 2: *In vitro* Antibacterial activity of various extracts (Hexane, Chloroform, Methanol and Ethyl acetate) of *Volvariella bombycina*

	Extract			
Bacteria	Hexane	Chloroform	Methanol	Ethyl acetate
	Zone of inhibition (mm)*			
Bacillus subtilis	13	14	15	17
Staphylococcus aureus	10	11	14	16
Escherichia coli	10	10	12	16
Klebsiella pneumoniae	8	9	12	14
Pseudomonas aeruginosa	-	-	8	9

*Values are mean of three replicates; '-': no zone of inhibition

inactive against the gram negative bacteria *P. aeruginosa*. *B. subtilis* was the most susceptible gram-positive bacteria followed by *Stap. aureus*. *P. aeruginosa* was the most resistant gram-negative bacterial strain followed by *K. pneumonia* and *E. coli*.

DISCUSSION

The natural origins of human foods are biologically diverse, ranging widely in texture and composition. The quality of food is based on the natural composition, the balance between the nutrient and the anti-nutrient composition. It is usually a concern over the chemical composition or contamination of food and the effect this has on its value to the consumer that generates the need for proximate analysis.

Proximate analysis of *V. bombycina* (dry powder) found to vary in mycelia and fruiting body [6]. Results revealed that *V. bombycina* is rich in protein with low lipid content make *V. bombycina* is an ideal food (Table 1). Ash content showed *V. bombycina* is rich in minerals with less soil contaminant. The moisture content in *V. bombycina* suggests that care must be taken in their handling and presentation as high moisture contents promote susceptibility to microbial growth and enzyme activity. The presence of crude fiber content in the *V. bombycina* will help to promote good digestion. The overall result from proximate analysis showed *V. bombycina* is an ideal food for a good health.

In the present study crude extracts of *V. bombycina* from polar to less polar organic solvent were tested against the clinically important bacterial isolates. Our study showed that ethyl acetate extract was certainly much better and powerful (Table 2). This may be due to the better solubility of the active components in organic solvent [7]. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. It has been proposed that the mechanism of the antibacterial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells [8]. The growth media also seem to play an important role in the determination of the antibacterial activity. Based on the report by Lin *et al.* Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study [7]. Amongst the Gram-positive and Gram-negative bacteria, Gram-positive bacterial strains were more susceptible to the extracts as compared to Gram-negative bacteria. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria rather than Gram-negative bacteria [9]. This may be due to the presence of double layers of the cell wall in Gram negative cells protect the cytoplasm better than the single layer in Gram positive cells.

The results from present study supported the usage of the *V. bombycina* mushroom as an ideal food and suggested that studied mushroom extracts possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. This study also recommended that agricultural system in the India region be encouraged to domesticate this healthy food.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R. Sheela Devi, Associate Professor Department of Physiology, University of Madras, Chennai, Tamilnadu, India.

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