

Original Research Article

Comparison of Nutrient Contents and Antimicrobial Properties of *Pleurotus djamor*, *Agaricus bisporus* and *Ganoderma tsugae*

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ABSTRACT

Keywords

Mushroom,
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The edible mushrooms of *pleurotus djamor*, *Agaricus bisporus* and non-edible mushroom *Ganoderma tsugae* were used for in this study. The dry weight, nutrient contents and antimicrobial activity was studied in edible and non-edible mushrooms. The dry weight of the mushroom was analysed and it was found in the range of 11-16 gm/100gm.the maximum dry weight observed in *Ganoderma tsugae* (16.1 gm/100gm) followed by *Agaricus bisporus* (14.3 gm/100gm) The maximum nutrient content was observed in *Agaricus bisporus* and the minimum amount of nutrient content was observed in *Ganoderma tsugae*. The maximum amount of protein (32.0 mg/gm), glucose (13.2 mg/gm) and free amino acid (5.2 mg/gm) content was observed in the *Agaricus bisporus* and the trace amount of was observed in *Ganoderma tsugae*. The antimicrobial activity was studied by the mushroom extracts (acetone and dimethyl sulfoxide) of *Pleurotus djamor*, *Agaricus bisporus* and *Ganoderma tsugae* against the pathogenic bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. The acetone extracts of *Agaricus bisporus* reported highest zone of inhibition (13 mm and 14 mm) against *Escherichia coli* and *Pseudomonas aeruginosa*. The *Agaricus bisporus* reported more antimicrobial activity in acetone extract and the *Ganoderma tsugae* reported more antibacterial activity in dimethyl sulfoxide extracts.

Introduction

Mushrooms are important constituents of this biosphere that is rich in cellulose is present. Mushrooms are naturally grown on the trunks, leaves, roots of trees as well as decaying woody materials and dead wood trees (Lindequist *et al.*, 2005). Mushrooms offer tremendous applications

as they can be used as food and medicines besides their key ecological roles. Mushrooms have been found effective against cancer, cholesterol reduction, stress, asthma, allergies and diabetes (Bahi, 1983). The carbohydrate content of mushrooms represents the bulk of fruiting

bodies accounting for 50 to 65% on dry weight basis. Protein is an important constituent of dry matter of mushrooms. Mushrooms are very useful for vegetarian food because they contain some essential amino acids which are found in animal proteins (Verma *et al.*, 1987).

Edible mushroom characteristically contain many different bioactive compounds such as polysaccharides, glycolipids, sesquiterpenes etc., with diverse biological activities such as anticancer, antibacterial, antifungal and antiviral agents. *Ganoderma lucidum* is one of the most famous traditional chinese medicinal herb which has antimicrobial effects due to the extracts derived from this mushrooms which contain bacteriolytic enzyme and acid protease (Klaus and Miomir, 2007). Smania *et al.* (2007) observed high zone of inhibition of in methyl extract form *G. lucidum* against *Escherichia coli* and *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* while least zone of inhibition was recorded against *Bacillus species*.

Extracts of *Ganoderma applanatum* (Smania *et al.*, 1999) and *Ganoderma pfeifferi* (Mothana *et al.*, 2000) have been shown to possess significant antimicrobial activity against *E. coli*. Sheena *et al.* (2003) reported that methanol extract of *Ganoderma lucidum* showed remarkable antimicrobial activity against *Escherichia coli*, *Salmonella* sp., and *Bacillus subtilis*. Keypour *et al.* (2008) investigated the antimicrobial activity in chloroform extract of *G. lucidum* against *Bacillus subtilis* and *Staphylococcus aureus*.

The present study deals with collection, identification, studies of nutrient contents and antimicrobial activities of selected mushrooms.

Materials and Methods

The present work deals with the nutrient contents and antimicrobial activity in the edible and non-edible mushrooms.

Collection of Mushroom

The three types of edible and non-edible mushrooms are selected for our study. The oyster mushroom (*Pleurotus djamor*) (MDU-1) was obtained from Ayya Nadar Janaki Ammal College Mushroom Centre. The Button mushroom (*Agaricus bisporus*) procured from supermarket in Sivakasi. The non-edible mushroom *Ganoderma tsugae* were collected in Ayya Nadar Janaki Ammal College campus during rainy season (Plate 1).

Identification of Mushroom

The collected mushroom were identified by the help of morphological characters of the colour size, shape, nature of pileus, stipe and gills with the help of Mycology penned by Alexopoulos (1996). The above three types of mushroom were subjected for the analysis of dry weight, nutrient content and antimicrobial properties.

Preparation of Mushroom Powder

The collected mushrooms of *Pleurotus djamor* and *Agaricus bisporus* were dried in hot air oven at 50°C for 24 hours. Then the dried mushroom were ground and powdered by using mixie. The non-edible mushrooms of *Ganoderma* were kept in the hot air oven at 60°C for one week and then it was powdered by grinding.

Dry Weight

Fresh mushrooms of (100 mg) *Pleurotus djamor*, *Agaricus bisporus* and

Ganoderma tsugae was weighed separately and it was kept in the hot air oven at 80°C for two days. The dry weight of the mushroom was calculated by weighing the mushrooms in an electronic balance separately.

Estimation of protein

Fresh mushrooms (100 mg) were homogenized in 10 ml distilled water and the extract was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and added with 1 ml of 20% TCA and it was kept for half an hour. Then centrifuged at 5000 rpm for 5 minutes. The pellet was washed with twice the volume of acetone and it was centrifuged. The supernatant was discarded, and the pellet was mixed with 5 ml of 0.1N NaOH and it was used as test solution. The test solution was quantitatively measured by taking 0.5 ml of test solution, 4 ml of distilled water, 5 ml of alkaline copper reagent and 0.5 ml of Folin phenol reagent in test tubes. Blank was also prepared by taking 4 ml distilled water, 5 ml of alkaline copper reagent and 0.5 ml of Folin phenol reagent. The absorbance was noted at 650 nm using ELICOSL 171 spectrophotometer. The protein content was estimated by using protein standard graph.

Estimation of Glucose

Fresh mushrooms (500 mg) were homogenized with 10 ml of distilled water using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and it was added with 1 ml of 20% TCA and it was kept for 30 minutes, then centrifuged at 5000 rpm for 5 minutes. The supernatant of test solution was taken

and add with 4 ml of anthrone reagent slowly to the test tube. The supernatant was collected and it was made upto 100 ml with distilled water. Later it was added with 0.9 ml of distilled water. Then it was thoroughly mixed by using cyclomixture. Later the contents were boiled in water bath for 10 minutes by placing the marbles on the mouth of test tube. After 10 minutes of boiling, the test tubes were allowed to cool down at room temperature and the absorbance was noted at 620 nm using ELICOSL 171 spectrophotometer. The blank was also prepared by taking 4 ml of anthrone reagent and 1 ml of distilled water. The glucose content was calculated with the help of glucose standard graph.

Estimation of free amino acid

Fresh mushrooms (100 mg) were homogenized in 10 ml of 70% ethanol using a mortar and pestle. The homogenate was filtered through single layer of cheese cloth and it was centrifuged at 3000 rpm for 5 minutes. The pellet was discarded and the supernatant was collected and it was used as test solution. 0.5 ml of supernatant, 0.5 ml of 70% ethanol was taken in a test tube and 2 ml of ninhydrin reagent was added. The tubes were kept in water bath for 10 minutes and then it was cooled. The blank was also prepared by taking 70% ethanol, ninhydrin. The absorbance was measured at 570 nm using ELICOSL 171 spectrophotometer. The quantity of free amino acid was estimated by using a standard graph of amino acid.

Antibiotic assay

Sources of microbes

Antibiotic assay was assessed by using two bacterial strains of *Escherichia coli* and *Pseudomonas aeruginosa* obtained

from Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi.

Assay of Antimicrobial activity

The antimicrobial activity was assessed by well diffusion method in the Muller Hinton Agar medium.

Preparation of mushroom extract

The dried mushroom powder was used for the preparation of mushroom extract. Five gm of mushroom powder was mixed with 100 ml of dimethyl sulfoxide and acetone separately in a beaker and it was placed on a shaker for 24 hours. The aqueous solutions were filtered through Whatman (No.1) filter paper and then it placed on the rotary evaporator vacuo, for 15 minutes at 37°C. Then the residue was dissolved with 100 ml of dimethyl sulfoxide and acetone separately and stored at 4°C for further analysis.

Assessment of antimicrobial activity

The Muller Hinton Agar medium was poured in an sterilized petriplates and then it was allowed for solidification. The selected microorganisms of (*Escherichia coli* and *Pseudomonas aeruginosa*) were swabbed over the medium using swab culture technique, in separate plates. The wells were prepared in the medium using sterile cork borer (5mm). The prepared extracts of dimethyl sulfoxide and acetone were loaded in different concentration of 100µl, and 150µl in an each well separately. The control plate was loaded with acetone and dimethyl sulfoxide solvent in each well separately. Later the plates were incubated at 37°C for 2 hours. After 24 hours, the zone of inhibition was measured in mm. The antibiotic assay was assessed separately in dimethyl sulfoxide and acetone extracts of mushrooms for

separate microorganisms.

Results and Discussion

The collected mushrooms were identified and the results of the nutrient contents and antimicrobial activities were given below. The three kinds of edible and non edible mushrooms *Pleurotus djamor*, *Agaricus bisporus* and *Ganoderma tsugae* were identified based on their characters.

Nutrient contents

The dry weight of the mushrooms was noted in the mushroom of edile and non-edible mushroom *Ganoderma* recorded maximum dry weight. The dry weight of mushrooms were (16.14gm, 14.34gm and 11.62 gm/100gm of fresh wt) in *Ganoderma tsugae*, Button mushroom and oyster mushroom respectively. The maximum dry weight of (16.14 g/100gm) was noted in the *Ganoderma tsugae* and minimum of (14.34g/100gm) was noted in Button mushroom. The dry weight content was less in the edible mushrooms of *Agaricus bisporus* and *Pleurotus djamor*. These edible mushrooms are very soft and rich water content than the *Ganoderma*. The *Ganoderma* fruiting bodies are very hard, thick and less water content. Therefore the dry weight of *Ganoderma* was more. The protein content was high in mushroom compared to vegetables. Soluble protein content of the mushrooms also varied with the edible and non edible mushrooms.

A very high amount of 32 mg/g fr .wt of soluble protein was recorded in Button mushroom. Samsheer *et al.* (2006) noted the high protein content in button mushroom. The protein content of the mushroom was in the range of 19-35% (Li and Chang, 1992). But the minimum amount of 28.0mg/g/fr.wt⁻¹ was noted in

oyster mushroom compared to *Ganoderma tsugae*. Wahid (1990) also found minimum amount of protein in *Ganoderma tsugae*. The protein content of non edible mushroom was 16.0 mg/g fr.wt⁻¹.

Soluble sugar content of the mushrooms were also varied with the edible and non edible mushrooms. The carbohydrate content of edible and non-edible mushrooms represents the range of 4-13% on dry weight basis (Florezak *et al.*, 2004). However the soluble glucose content was very less than the protein content. Table shows very high amount of 13.2 mg/g/fr. wt. of sugar was noted from the Button mushroom and minimum amount of 9.7 mg/g/fr. wt. of sugar in oyster mushroom. The non edible mushrooms reported 4.4 mg/g/fr. wt. the same trends results observed by Florezak *et al.* (2004). The mushrooms are also rich in amino acid. The amino acid contents were also varied with edible and non edible mushroom. However the amount of 5.15 mg/g/fr. wt⁻¹ free amino acid was noted from the button mushroom. Samsheer *et al.* (2006) also reported the same observation in Button mushroom. The minimum amount of 3.4 mg/g/fr. wt⁻¹ amino acid was noted in the oyster mushroom. The amino acid contents were in more mushrooms in when composed with other food stuffs. The amino acid content mushrooms obtained from *Ganoderma tsugae* is very less 1.29 mg/g/fr.wt⁻¹ (Table1). The same result observed by Chandalia *et al.* (2000).

Antimicrobial activity assessment

The selected microorganisms of *Escherichia coli* and *Pseudomonas aeruginosa* were cultured in slant culture methods which are used for antibiotic sensitivity test. The antimicrobial activity

was studied by well diffusion method by using the mushroom extracts extracted from acetone and dimethyl sulfoxide separately. Similar solvent extraction was also carried out by using the solvents of acetone, ethanol, methanol and ethyl alcohol by Dulger and Gonuz (2004) and Chloroform extract is also used by Keypour *et al.* (2008). The results of antibiotic assay at 100µl concentration, the maximum zone of inhibition (12mm) was observed in *Agaricus bisporus* (Plate 2a), where as the minimum zone of inhibition (10mm) was observed in *Ganoderma tsugae* against the pathogen *Escherichia coli* in acetone extracts. Smania *et al.* (2007) also observed antimicrobial activity by using the bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella aureus* and *Bacillus sp.*, in the extract of *Ganoderma lucidum*.

The maximum zone of inhibition (14 mm) observed in *Agaricus bisporus* whereas the minimum zone of inhibition (11 mm) was observed in *Ganoderma tsugae* against the pathogen *pseudomonas aeruginosa*. As per the results, the *Agaricus bisporus* shows more inhibitory zone than the offer two mushrooms (Plate 2b). The antibiotic assay at 150 µl concentration, the zone of inhibition was 13 mm in *Agaricus bisporus* where as the minimum zone of inhibition 11 mm in *Pleurotus djamor* against the pathogen *Escherichia coli*. However the zone of inhibition is 12 mm in *Ganoderma tsugae* against the pathogen *Escherichia coli*. The maximum zone of inhibition (15 mm) was found in *Agaricus bisporus*, whereas the minimum zone of inhibition (12 mm) has observed in *Pleurotus djamor* against the *Pseudomonas aeruginosa*. The *Agaricus bisporus* shows more inhibitory zone than the other two mushrooms. The inhibitory zone was also increased with increasing

concentration of extracts. However the acetone extract of *Agaricus bisporus* shows more inhibitory zone at 100 µl and 150 µl concentration against the pathogen *Escherichia coli* and *Pseudomonas aeruginosa* than other extracts. Vamanu *et al.* (2010) determined the antimicrobial activity against *Escherichia coli*, *Bacillus cereus* and *Listeria innocua* in the mushroom powder extract. In dimethyl sulfoxide extract, the antibiotic assay at 100 µl concentration, the maximum zone of inhibition 7mm was observed in *Ganoderma tsugae*, whereas the minimum zone of inhibition (5 mm) was observed in the other two mushrooms, against the pathogen *Escherichia coli*. The maximum zone of inhibition 8mm in *Ganoderma tsugae* whereas the minimum zone of inhibition 7 mm in the other two mushrooms against the pathogen *Pseudomonas aeruginosa* were recorded.

As per the results the *Ganoderma tsugae* shows more inhibitory zone than the other two mushrooms (Plate 3). The antibiotic assay at 150 µl concentration, the maximum zone of inhibition (8 mm) observed in *Ganoderma tsugae* where as the minimum zone of inhibition (6 mm) was observed in *Agaricus bisporus* against the pathogen *Escherichia coli*. The maximum zone of inhibition 13mm observed in *Ganoderma tsugae* where as the minimum zone of inhibition (10 mm) observed in *Pleurotus djamor* against the pathogen *Pseudomonas aeruginosa*.

The dimethyl sulfoxide of extract of *Ganoderma tsugae* shows more inhibitory zone at 100 µl and 150 µl concentration against the pathogen *Escherichia coli* and *Pseudomonas aeruginosa*. It was found out that all the three type of mushrooms reported the presence of antibacterial activity with various levels. It may be due

to the presence of phenols and tannins in the mushrooms. The zone of inhibition was more in acetone extracts when compared to dimethyl sulfoxide extracts of button mushroom against the selected microorganisms. High level of antibacterial activity was reported in *Agaricus bisporus* against *Pseudomonas aeruginosa* it is positively correlated with the findings of Kim and Fung (2004) (Table 2). It may be due to the presence of secondary metabolites and bioactive compounds in silver nanoparticles.

As per the results, it was found out that all the analysed mushroom extracts were reported antibiotic activity against *Escherichia coli* and *Pseudomonas aeruginosa* in well method using the solvent extracts of both acetone and dimethyl sulfoxide. The zone of inhibitions were also varied with mushroom extracts and type of microorganisms used. The active principles of secondary metabolites also varied with various types of mushrooms which inhibit the growth of bacteria. However the variations in the zone of inhibition were reported in acetone and dimethyl sulfoxide extracts due to the selective solubility of secondary metabolites in different solvents.

Metabolite such as phenolic compounds by most macro fungus has been responsible for their antimicrobial and antioxidant activity of mushroom (Gao and Tang *et al.*, 2005). This study has revealed that the edible and non-edible mushrooms exhibited various levels of antimicrobial activity in various solvents. The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harvested as potential antibacterial substances.

Table.1 Comparison of nutrient contents and antimicrobial properties of selected mushrooms

S. No	Mushrooms	Dry weight (mg/100gm Fr. wt ⁻¹)	Protein (mg/gm Fr. wt ⁻¹)	Glucose (mg/gm Fr. wt ⁻¹)	Amino acids (mg/gm Fr. wt ⁻¹)
1	<i>Pleutorus djamor</i>	11.6	28.0	9.2	3.4
2	<i>Agaricus bisporus</i>	14.3	32.0	13.2	5.2
3	<i>Ganoderma tsugae</i>	16.1	16.0	4.4	1.3

Table.2 Antibiotic assay (Well Method) zone of inhibition against selected microbes in the selected mushrooms using acetone and Dimethyl sulfoxide extract different concentrations.

Pathogens used	Zone of inhibition (Diameter in mm)						
	Concentration (µl)	<i>Pleutorus djamor</i>		<i>Agaricus bisporus</i>		<i>Ganoderma tsugae</i>	
		Acet one	Dimethyl sulfoxide	Acet one	Dimethyl sulfoxide	Acet one	Dimethyl sulfoxide
<i>Pseudomonas aeruginosa</i>	100	12	7	13	7	11	8
	150	12	10	14	11	13	13
<i>Escherichia coli</i>	100	11	5	12	5	10	7
	150	11	8	13	6	12	8

Plate.1 Selected edible and non-edible mushrooms a. *Pleurotus djamor*; b. *Agaricus bisporus* and c. *Ganoderma tsugae*



a



b



c

Plate.2 Inhibitory effect of *Agaricus bisporus* against the pathogen a. *E. coli*; b. *Pseudomonas aeruginosa*

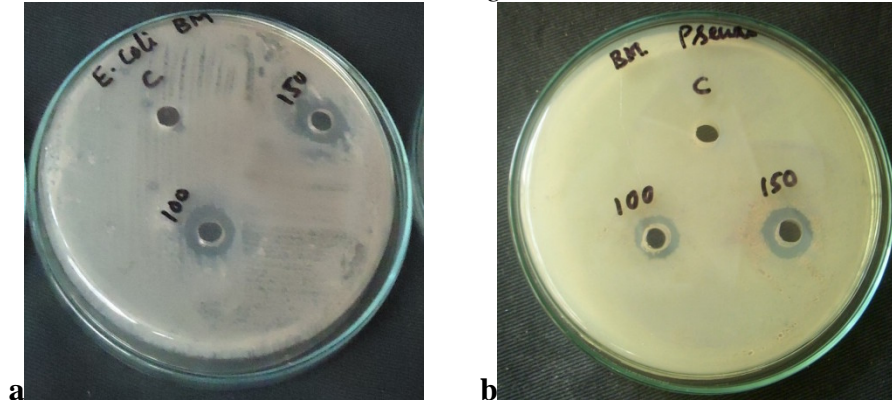
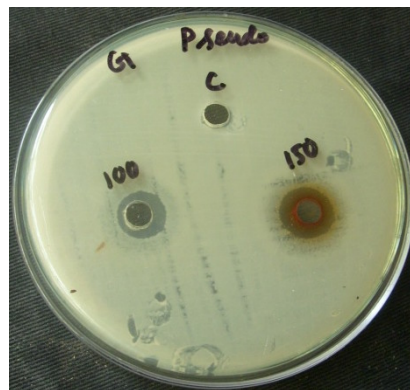


Plate.3 Inhibitory effect of *Ganoderma tsugae* against the pathogen *Pseudomonas aeruginosa*



References

- Alexopoulos, C.J., Mim, C.W, and Blackwell M, 1996. In *Introductory mycology* 4th edition Jhon wiley and sons. *New York*.
- Bahi, N (1983). Medicinal value of edible fungi. In; proceeding of the International Conference on Science and Cultivation Technology of Edible fungi. *Indian Mush Sci* 11, pp. 203 – 209.
- Chandalia, M, Garg, A, Lutjohann, D, Von Bergmann, K, Grundy, SM, and Brinkly LJ 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N. Eng. J. Med*, 342:1392 – 1398.
- Dulger, B. and Gonuz, A. 2004. Antimicrobial activity of certain plants used in Turkish traditional medicine. *Asian J. of Plant Sci.*, 3: 104 – 107.
- Florezak, J, Karmnska, A, Wedzisz, A., 2004. Comparision of chemical contents of the selected wild growing mushrooms. *Chem., Toksykol.*, 37 : 365 – 371.
- Gao, Y.H., W.B. Tang, H, Gao, E.Chan ,J.Lan and Li, X.T. 2005, Antimicrobial activity of medicinal mushroom *Ganoderma*. *Food Res. Int.*, 21:211 – 229.

- Keypour, S, Riahi, H, Moradli, MF, and Rafati, H 2008. Investigation of the antibacterial activity of a chloroform extract of Lingzhi or Reishi medicinal mushroom *Ganoderma lucidum* from Iran. *Int. J. Med. Mush.*, 10(4): 345 – 349.
- Kim, S. and Fung, D.Y.C., 2004. Antibacterial effect of water soluble arrowroot (*Puerariae radix*) tea extract on food borne pathogens in ground beef and mushroom soup, *J.Food protection*, 67: 1953 – 1956.
- Klaus A., and Miomir N: 2007 influence of the extracts isolated from *Ganoderma lucidum* on some microorganisms. *Proceedings of National Science*, 113: 219 – 226.
- Li, G.S.F. and Chang. 1992. Nutritive value of *Volvariella volvaceae*, In: *Tropical Mush.*, pp 199-219.
- Lindequist, U., Niedermeyer, T.H.J. and Julichs, W.D. 2005. The pharmacological effects of mushrooms evidence based complementary and alternative medicine. *J. Med.* 2: 285 – 299.
- Monthana, R.A.A, Jansen, R, Julich, W.D. and Lindequist, U., 2000. *Ganomyacin* A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pffiferi*. *J. Nat. Prod.*, 63(3): 416 – 418.
- Samsher, G ,and Subbulakshmi, 2006. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chem.*, 89: 599-603.
- Sheena, N., Ajith, T.A., Mathew, A., and Janardhanan, K.K, 2003. Antimicrobial activity of *Ganoderma lucidum*, *Naverspours floccosa* and *Phellinus rimosus* occurring in South India. *J. Pharm. Biol.*, 41(8): 564 – 567.
- Smania, A., Delle, M.F., Smania, E.F.A. and Cuneo R.S. (1999). Antimicrobial activity of steroidal compounds isolated from *Ganoderma applanatum*. *Int. J. Med. Mush.*, 1: 325 – 330.
- Smania, E.F.A, Delle, M.F., Yunes, R.A. and Smania, R.A. 2007. Antimicrobial activity of methyl acetate from *Ganoderma australe*. *Brazillian J. of Pharm.*, 17(1): 14 – 16.
- Vamanu, E., Vamanu, A. and Pelinescu, D. 2010. Synthesis and partial Biochemical Characterization of Bacteriocin produced by *Lactobacillus paracasei*, *Chem.*, 61 (5): 502 – 505.
- Verma, R.N., Singh, G.B. and Bilgrami, K.S. 1987. Fleshy fungal flora. India – Manipur and Meghalaya. *Indian mush. Sci.*, 2: 414 – 421.
- Wahid, M. 1990. Nutritive value of wild edible mushrooms. *Int. Mush J. Tropics*. 10:59-64.