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The Effectiveness β-glucan Of Shiitake Mushrooms and *Saccharomyces cerevisiae* as Antidiabetic and Antioxidant in Mice *Sprague Dawley* Induced Alloxan

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Abstract. The shiitake mushrooms (*Lentinula edodes*) contain biologically active polysaccharides that mostly belong to the group of β-glucans. Beta-glucan was reported as cell wall components of bacteria, fungi, and yeast. Beta-Glucans naturally contain polysaccharides which can prevent Colorectal cancer, coronary heart disease, high blood glucose levels, insulin resistance, high cholesterol levels, and gut microflora. The aim of the study was to examine the antidiabetic and antioxidant activity of β-glucan from *Shiitake* mushrooms and *S. cerevisiae*. The experiment used a glucose tolerance test method to measure a decrease in blood glucose. Thirty one white male mice *Sprague Dawley's* induced by Alloxan were divided into 7 groups, each group was given 4 replications. Group I was normal control (untreated mice); group II was negative control (mice were given alloxan at a dose of 80 mg/kg bw and CMC Na); group III was positive control of diabetes (mice were given alloxan at a dose of 80 mg/kg bw and a dose of 5 mg/kg bw); group IV was antioxidant control group (mice were given alloxan at a dose of 80 mg/kg bw and vitamin E); in group V, VI and, VII, mice were given an alloxan at a dose of 80 mg/kg bw and each 50 mg/kg bw β-glucan shiitake, commercial β-glucan and β-glucan *S. cerevisiae*). Tests were conducted from 14 days in mice DM. The treatment of beta-glucan of shiitake mushrooms, commercial beta-glucans, and beta-glucan of *S. cerevisiae* showed a decrease in blood glucose levels of 55.87%, 32.31% and 58.0% respectively. The antioxidant activity test using the DPPH method showed that IC_{50} were 64.31 ug / ml, 78.83 μg/ml, and 60.61 ug/ml respectively.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease caused by the inability of body to produce insulin hormones as needed or caused by ineffective use of insulin. The disease is characterized by high blood sugar levels or hyperglycemia [1]. DM sufferer with uncontrolled hyperglycemia conditions will experience a degenerative process. Such conditions can cause damage to brain tissue and nerves, namely as a result of increased oxidative stress and decreased antioxidant defenses [2].

Basically there are two management of diabetes. The first is management without drugs through diet and exercise. The second is treatment with drugs. If the management of therapy without drugs does not succeed in controlling the sufferer's blood glucose levels, drug therapy needs to be done, either in the form of oral hypoglycemic drug therapy, insulin therapy, or a combination of both.

The use of herbal medicines for the treatment of diabetes mellitus has received great attention throughout the world. WHO also recommends it, especially to countries where access to treatment for diabetes is inadequate. The need of the use of natural products is increasing; it is related to the side effects of using insulin and oral hypoglycemic agents. Researches for the development of oral hypoglycemic drugs for diabetes have been carried out, which involved various sources of natural ingredients such as oyster mushrooms, shiitake mushrooms, straw

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mushrooms, and *Saccharomyces cerevisiae* containing beta glucan. Shiitake mushrooms (*Lentinula edodes*) produce β(1-3)(1-6)-D-glucans having anti-tumor effects [3].

S. cerevisiae widespread in nature is a potential strain of beta-glucans, because most of the cell walls are composed of β-glucans. B-Glucans are polymers composed of glucose monomers found in the cell walls of bacteria, fungi, yeast, algae, mosses, and plants. The most active form of beta-glucan consists of the D-glucose unit with a bond (1.3) with a side chain in position (1.6) [4]. β-glucan has the potential for treatment of diabetes and cardiovascular risk [5].

β-Glucan isolated from *Saccharomyces cerevisiae* could significantly reduce blood glucose levels. The percentage of the decrease of glucose levels in blood increased along with the addition of beta glucan doses given to diabetic mice. The Beta Glucan doses tested were 30, 60, 120 and 240 mg/kg. The percentage of the decrease of glucose levels in blood was respectively 33.5%, 35.0%, 35.7% and 38.7%. The standard anti-hyperglycemic drug, glibenclamide, was used as a comparison. The given dose, 10 mg/kg BB, could reduce blood sugar in mice by 40.0% [6]. B-glucan contained in wheat had the potential to prevent obesity and for prevention and treatment of type II diabetes mellitus [7].

Based on the description above, this study aims to examine the potential of shiitake mushroom β-glucan (*Lentinula edodes*), commercial beta glucan and beta glucan extracted from *Saccharomyces cerevisiae* at a dose of 50 mg/kg as antidiabetic and antioxidants in alloxan-induced mice. Alloxan has been found as an inducer of free radicals and a cause of degeneration [8].

EXPERIMENTAL DETAILS

Test Material

Shiitake mushroom (*Lentinula edodes*), commercial β-glucan (Takeda) and *Saccharomyces cerevisiae* culture were obtained from the Research Center for Biotechnology, LIPI. Animals of male white mice (*Rattus novergicus*) with a body weight of 160-200g were obtained from the Faculty of Veterinary Medicine, Bogor Agricultural Institute.

Extraction of β-glucans from Shiitake Mushrooms (*Lentinula edodes*)

A total of 20 g of Shiitake mushroom flour was added to 200 ml of water and heated over a waterbath for 1 h. The sample was cooled and ethanol 96% (1:1) was added. The mixture was centrifuged at 6000 rpm/min for 10 min at 4 °C. The formed pellets were heated for 10 min and cooled, then centrifuged at 6000 rpm/min for 15 min at 4 °C. Then, 95% ethanol (1:1) was added and it was left for 18 h at 4 °C. The solution was centrifuged again under the same conditions. The obtained pellet was added with Phosphate Buffer Saline (PBS) solution, rinsed with distilled water [9].

S. cerevisiae Aquaculture in Liquid GYP Media

A total of two fresh *S. cerevisiae* cultures were inoculated into 20 ml of liquid GYP medium, then incubated in a incubator shaker at 30 °C for 48 h at a speed of 150 rpm [10].

Morphological characteristics of *S. cerevisiae*

A total of 2 drops of fresh *S. cerevisiae* culture were dropped on the glass preparation. Then it was fixed by heating, given methylene blue coloring, and left for 5 min until the substance was absorbed completely then rinsed with water and aerated. Observation was carried out under the microscope with a magnification of 400 times.

B-glucan extraction from *Saccharomyces cerevisiae* Cell Culture

The 5-day-old *S. cerevisiae* culture was centrifuged at 6000 rpm at 30 ˚C for 15 min, then the supernatant was removed. Biomass was hydrolyzed with 0.75 M sodium hydroxide in a water bath for 6 h at 75°C. Then it was centrifuged at a speed of 6000 rpm at 30 °C for 15 min, then the supernatant was removed. The collected deposits were washed with 0.5 M acetic acid, then centrifuged at 6000 rpm at 30 °C for 15 min, and the supernatant was removed. The washing was repeated for three times. The precipitate was then rinsed with water and ethanol, then centrifuged at a speed of 6000 rpm at 30 °C for 15 min, a separate supernatant was removed [11].

Glucose Levels Using Phenol Sulfate Method

The standard stock solution of 1000 bpj glucose was respectively made into standard detretion of 20, 40, 60, 80, 100 bpj. A total of 0.5 ml of each standard series and β glucan samples were pipetted into the test tube and 0.25 ml of 5% phenol was added. The solution was added with 1.25 ml of concentrated H2SO4 and then it was stirred until homogeneous for 10 min. Uptake was measured by a spectrophotometer at λ 490 nm. Blank solution used distilled water as a substitute for the sample [12].

Protein Levels Using the Lowry Method

The standard series of standard stock solution of Bovine serum albumin (BSA) protein 1000 bpj was made in a 20, 40, 60, 80, 100 bpj respectively. A total of 0.5 ml of each standard series and β glucan sample was added with 0.5 ml of 1N NaOH. The solution was boiled for 20 min in a water bath, then cooled. The solution was added with 0.5 ml of Lowry reagent and 0.5 ml of folin C solution, then let stand for 30 min. The solution was measured by a spectrophotometer at λ750 nm. Blank solution used distilled water as a substitute for the sample [13].

Effectiveness Test of Beta Glucose as Antidiabetic

Test animals were divided randomly in 7 groups, each consisted of 4 mice. After being adapted for a week, the mice were fasted for 10 hours and their blood was taken to determine the initial blood glucose level. Furthermore, the mice were induced intravenously with alloxan monohydrate 80 mg/kgBW [14]. Post-induction, the alloxan of blood glucose levels in mice was measured again. The increase of the mice's blood glucose levels reached ± 200 mg/dL or more; so, mice were considered to have diabetes.

The distribution of treatment groups was done as follows. In group I, normal control, mice were not given induction treatment and test material. In group II, negative control, mice were given CMC-Na 0.5%. In group III, positive control of diabetes, mice were given Glibenchlamide 5 mg/kgBB. In group IV, positive antioxidant control, mice were given vitamin E 10 mg/kgBB. In group V, mice were given Shiitake mushroom-glucan extract 50 mg/kgBB. In group VI, mice were given commercial β-glucan (Takeda) 50mg/kgBB. In group VII, mice were given *S. cerevisiae* β-glucan extract 50 mg/kgBB. After 14 days of treatment, mice's blood glucose levels were measured again. Blood samples obtained from the seven groups of mice were tested for antidiabetic with an easy touch glucometer, and antioxidant tests were carried out with the DPPH method to determine β-glucan free radical capture activity from shiitake, commercial β-glucan, and β-glucan *saccharomyces cerevisiae* in mice induced with alloxan.

Antioxidants Activity of Beta Glucan Test

Making Test Solution

The blood serum of mice from each treatment group was dissolved in pro-analysis methanol to obtain concentrations of 25, 50, 75, 100 ppm respectively. Test of antioxidant activity with DPPH (1,1-diphenyl-2-picrylhydrazyl [15].

A total of 1 mL of blood serum samples in methanol with a concentration of 25, 50. 75, 100 ppm was added to 2 mL DPPH 0.1 mM (Sigma-Aldrich). The mixture was then shaken and incubated at room temperature for 30 minutes in a dark place. The absorbance of solution then was measured at λ max 516 nm. The same treatment was also carried out for blank solutions (DPPH solution which did not contain test material) and positive control of

vitamin E with concentrations of 4, 6.8 and 10 ppm. The blank solution consisted of 2 mL DPPH 0.1 mM and 1 mL methanol p.a. The percentage of antioxidant activity of the data resulted from the absorbance measurements was analyzed using the following equation:

$$\% \text{ Inhibition } = \frac{absorption\ of\ sample - absorption\ of\ blanks}{absorption\ of\ blanks} \text{ x } 100\%$$

Statistical Analysis

The ANOVA test aimed to determine whether there were significant differences in blood glucose levels due to treatment. The results of ANOVA analysis showed $p < 0.05$, which showed that there was a significant difference in the treatment group. The data was then analyzed by Duncan Test which aimed to determine the differences between each treatment group.

RESULTS AND DISCUSSION

B-Glucan Extract from Shiitake Mushrooms

Shiitake mushroom beta glucan extraction was carried out by maceration method. As many as 20g of dried shiitake mushrooms (*lentinula edodes*) was boiled with 200 ml of water for 2 h. Beta glucan extraction was carried out based on the differences in its solubility. Some beta glucans was dissolved in water (*soluble*) and some was insoluble in water [16]. Some beta-glucans that was not soluble in water could be dissolved in alkali. Glucan is a heterogeneous group of glucose polymers, consisting of β- (1,3) β-D-glucopyranosyl units with β- (1.6) side chains and varying lengths. The extraction of β- (1,3); β- (1,6) -D-glucan compounds by hot aquades could happen because the heating process made the branch chains of β- (1,3); β-(1,6)-D-glucans open. The condition allowed aquadest to come into the structure of the polysaccharide β- (1,3); β-(1,6)-D-glucan and hydrogen bonds interacted with OH groups of compounds β- (1,3); β- (1,6)-D-glucan. Therefore, β- (1,3); β-(1,6)-D-glucan could be extracted into aquadest solvents. Within the process, gelatinization happend which could be seen from the thickened extraction supernatant. The average yield obtained from 20 grams of shiitake mushrooms (*lentinula edodes*) carried out with three replications was 1.27%.

Morphology of *Saccharomyces cerevisiae*

Observation of *S. cerevisiae* cells with methylene blue staining was carried out to ensure that the yeast to be used was not contaminated by other microorganisms. The observations under the microscope with a magnification of 400× can be seen in Fig. 1 below.

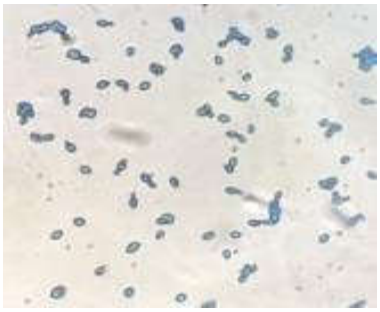

FIGURE 1. Observation of cell morphology of *S. cerevisiae* SAF with 400x magnification

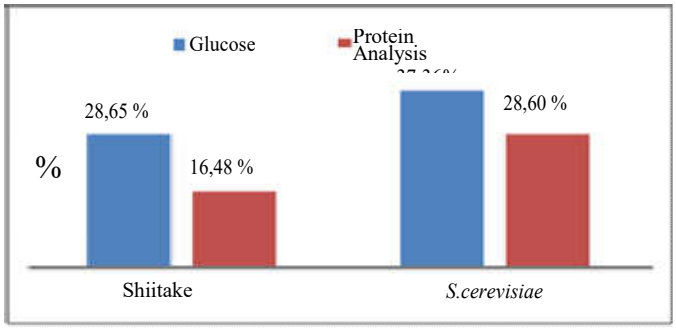

FIGURE 2 . Bar Diagram of the result percentage of Glucose and Protein Levels of Shiitake Mushrooms (*Lentinula edodes*) and *S. cerevisiae*

B-Glucan Extract from *Saccharomyces cerevisiae*

The structural components of the *S. cerevisiae* cell wall were Glucan β-1.3 and β-1.6, chitin, and manoprotein which are covalently bound. Glucan β-1.3 was synthesized through complex enzymatic reactions [17] . The results of beta glucan extraction from *S. cerevisiae* are listed in Table 1 below.

TABLE 1. Dry weight of cell biomass and β-Glucan *S. cerevisiae*

GYP Media Repetition (100 ml)	Dry weight of cell biomass of *S. cerevisiae* (g) ± SD	Dry weight of Beta glucan (g) ± SD
1	1,4835+0,2441	0,0529+0,0111
2	1,1763+0,0654	0,0523+0,0107
3	1,7968+0,1956	0,0916+0,0113

In the production of β-glucans it was expected that the protein content is low. Fig. 1 shows that Shiitake mushroom glucan beta glucose level was 28.65% and protein content was 16.48%. Glucose glucose level of *S. cerevisiae* was 37.36% and protein content was 28.60%. The results indicated that the lower the protein content, the higher the purity of β-glucan.

Induction of diabetes in mice in this study used the method of pancreatic destruction by giving diabetonics, i.e. alloxan. Alloxan used in the study was alloxan monohydrate at a dose of 80 mg/kg carried out intravenously [18]. The results of the analysis of mice blood with glucose levels above 200 mg/dl declared that the mice had diabetes and could be used as experimental animals to test antidiabetic compounds. The condition of hyperglycemia was caused by the fact that alloxan specifically accumulated in beta cells. Alloxan could cause intracellular calcium homeostatic disorders by increasing the concentration of cytosolic free calcium ions in the beta cells of the pancreatic Langerhans island which further opened the calcium canal depending on the voltage. More input of calcium ions into cells made insulin concentration increase very rapidly, and significantly resulted interference in peripheral insulin sensitivity in a short time. Hypoglycemia was a characteristic of diabetes and this phase lasted approximately 3 h. It showed that alloxan-induced hyperglycemia in mice was followed by severe and fatal hypoglycemia, and after duration of several hours it resulted hyperglycemia. The final phase of the blood glucose response to alloxan was referred to as permanent diabetes, the hyperglycemic phase happened between 24 and 48 h after alloxan [19].

Observation was carried out after mice had diabetes due to alloxan induction and 14 days after the test material treatment. Taking blood was carried out on the 15th day after treatment, then mice were sacrificed to take blood serum. The control treatment was carried out by giving CMC Na 0.5%. Observation was carried out every 1 week during the treatment, to ensure that the effects of alloxan were not lost during the 14-days treatment. It is due to the fact that the digestive system of mice did not have cellulose enzymes; so, the use of CMC wolud not affect blood glucose levels.

TABLE 2. Average rat blood glucose levels of Pre-Induction, After Induction, and After Treatment

Group	Treatment	Blood Glucose Level			Decrease Glucose Level (%)
		Pre-Induction Day-1	After Induction Day-3	After Treatment Day-14	
I	Normal Control	97.5	102.5	112.0	-
II	CMC Na 0.5% (Negative Ctrl)	97.75	353.0	340.0	3.68
III	Glibenchlamide (Positive Ctrl)	99.0	407.0	115.7	71.74
IV	β- glucan Shiitake Mushroom	99.75	315.0	139.5	55.87
V	β- glucan Commercial	102.75	328.5	222.5	32.31
VI	β- glucan *S. cerevisiae*	101.25	350.75	147.5	58.00

Table 2 shows the results of the average blood glucose level of mice after treatment for 14 days. The results indicated that the CMC Na 0.5% treatment decreased blood glucose levels in mice. It was presumably due to the effect of alloxan which only caused acute type diabetic mice, this type of acute diabetes caused the effects of unstable alloxan.

The decrease in blood glucose levels in negative controls with CMC was 3.68%. The result was lower than the decrease in glucose levels in the glibenclamide treatment which was 71.74%. Decreased blood glucose levels due to

glibenclamide treatment had an effect on mice's blood glucose levels at all times of observation. Glibenchlamide is an oral antidiabetic drug in the sulfoniurea group with a working mechanism that stimulates insulin secretion from the granules of pancreatic β-langerhans cells.

All of the treatment group of shiitake mushroom β-glucan extract, commercial β-glucan group, and β-glucan extract of *S. cerevisiae* at a dose of 50 mg/kg at the end observations showed a different reduction percentage in blood glucose levels. The treatment of β-glucan extract from Shiitake Mushrooms showed a percentage decrease of 55.87%; the commercial β-glucan treatment showed a decrease in percentage of 32.31%; and the *S. cerevisiae* b-glucan treatment showed a percentage decrease of 58.0%.

Blood glucose level data after induction of alloxan showed a normally distributed distribution tested by Shapiro-Wilk: $p > 0.05$. Data homogeneity test results, $p > 0.05$, showed homogeneous data; then, it was analyzed by parametric 1-factor analysis of variance (ANOVA) test. The most effective source of β-glucan to reduce blood glucose levels, in this study, was β-glucan from *S. cerevisiae* at a dose of 50 mg/kgbb. The results showed a percentage decrease in blood glucose which was not significantly different from the treatment of glibenchlamide.

B-glucan doses of 50 mg/kg of Shiitake Mushroom (*Lentinula edodes*) and commercial beta-glucans could reduce blood sugar levels which are not significantly different from beta-glucans from *S. cerevisiae*. The ability of β-glucans in reducing blood glucose levels in diabetic mice was related to the activity of β-glucans in reducing blood glucose by delaying emptying the stomach; so, the glucose diet was absorbed more gradually. After β-glucans were given orally, glucose levels could decrease in 14 days. Thus, β-glucans could reduce appetite and reduce food intake [5].

β-Glucan was resistant to acid so it could pass through the stomach. Macrophages in the mucosal lining of the intestinal wall took β-glucan particles through the β-glucan receptor. Activation of cells was part of their natural antigen-presenting function to release cytokines and induce activation of systematic immunity. β-Glucan could also reduce blood cholesterol by preventing the absorption of cholesterol from food in the stomach and intestines. In addition, β-glucans have been proven to reduce LDL cholesterol and increase HDL, which might alleviate insulin resistance [4].

Different beta-glucan effects were caused by the different characterization of the structure of beta-glucans. It could have an effect on reducing lipid and antidiabetic effectiveness. β -glucan shiitake mushrooms and *S. cerevisiae* had β-(1.3) and β- (1.6)-glucoside bonds, which became the main components of the polysaccharide. Therefore, it caused a decrease which was not significantly different between shiitake mushroom b-glucan and *S. cerevisiae*; the different physiological conditions of the individual mice could also affect the differences in the effects of the β-glucan therapy produced. Therefore, there were differences in therapeutic effects between extracts of β-glucans derived from shiitake mushrooms (*Lentinula edodes*), *S. cerevisiae*, and commercial beta-glucans.

Testing Beta Glucan as an Antioxidant

Free radicals that are commonly used as models in the study of antioxidants or free radicals were 1,1-diphenyl2-picrylhydrazyl (DPPH). The DPPH method was a simple, fast, and easy method for screening free radical capture activities. The test results for antioxidant activity of vitamin E, β-glucan extract from shiitake mushrooms (*Lentinula edodes*), commercial β-glucan, and β-glucan *S. cerevisiae* can be seen in (Table 3.)

The results of the antioxidant activity test using the DPPH method showed that in the Shiitake Mushroom β-glucan treatment sample, IC_{50} of 64.31 µg/ml was obtained; the sample of IC_{50} β-glucan treatment was 78.83 µg/ml; and β-glucan (*S. cerevisiae*) treatment samples IC_{50} was 60.61 µg / ml. IC_{50} value was an effective concentration value, which was the value that showed the concentration of the test material (µg/ml) which could inhibit the oxidation process by 50%.

The mechanism given by β-D-glucan in antioxidant immersion activity was related to anomeric hydrogen activity, the mechanism of gamma irradiation antioxidant β-D-glucan was associated with the formation of carbonyl groups that could interact with transition metal ions such as Cu^{2+} or Fe^{2+} . In the lipid system, iron complexes with carbonyl groups were formed in β-D-glucans which were irradiated. It affected lipid protection and decreased the lipid peroxidation process. The more the number of carbonyl groups formed in β-D-glucan which was irradiated, it could result the protection of lipid peroxidation [20].

Table 3. Test results of antioxidant activity of vitamin E and blood serum by treatment of shiitake mushroom, β-glucan, beta glucan extract, and *S. cerevisiae* β-glucan extract.

No	Sample Treatment	Concentration (ppm)	Absorbance (nm)	% inhibition	IC_{50} (µg/ml)
1	Blank	0	0.598	-	
2	Vitamine E	4	0.497	16.88	
		6	0.454	24.08	12.80
		8	0.395	33.94	
		10	0.368	38.46	
3.	β- glucan	25	0.340	43.14	
	Shiitake Mushrooms	50	0.309	48.32	64.31
		75	0.289	51.67	
		100	0.249	58.36	
4.	β- glucan commercial	25	0.369	38.29	
		50	0.350	41.47	79.83
		75	0.301	49.66	
		100	0.271	54.68	
5	β- glucan	25	0.340	43.14	
	S. cerevisiae	50	0.309	48.32	60.61
		75	0.289	51.67	
		100	0.249	58.36	

Data from mice blood serum analysis obtained from the β-glucan treatment group from Shiitake Mushroom, Commercial beta-glucan group, and *S. cerevisiae* beta-glucan group had strong antioxidant activity (Table 3). The division of categories as antioxidants was very strong if the IC_{50} value was less than 50 µg/ml; the strong category for IC_{50} was worth 50-100 µg/ml [21]. The results indicated that from the three different sources of beta-glucans there were also different effects of therapy.

SUMMARY

The administration of shiitake mushroom β-glucan extract (*Lentinula edodes*), commercial β-glucan, and *S. cerevisiae* β-glucan extract at a dose of 50 mg/kgbb could reduce blood glucose levels in alloxan-induced mice with a decrease percentage of 55.87 %, 32.31%, and 58.0%. The administration of shiitake mushroom β-glucan extract (*Lentinula edodes*), commercial β-glucan, and *S. cerevisiae* β-glucan extract had antioxidant activity. The most effective IC_{50} value of *S. cerevisiae* β-glucan extract was 60.61 µg/ml.

REFERENCES

1. A. Sutandi, "Self Management Education (Dsme) Sebagai Metode Alternatif Dalam Perawatan Mandiri Pasien Diabetes Melitus Di Dalam Keluarga," Thesis, STIKES Binawan, 2012.
2. F. Giacco and M. Brownlee, Circ. Res. **107**(9), 1058-70 (2010).
3. H. Crespo, L. P. Maiso, H. Guillen and C. G. Amebola. Food Nutr. Res. **61**(1), 1412791(2017).
4. S. Rahar, G. Swani, N. Nagpal and G. S. Singh, Jour. Adv. Pharm. Tech. Res. **2**(2), 94-103 (2011).
5. J. Chen and K.Raymond. Vasc. Health Risk. Manag. **4**(6), 1265–1272 (2008).
6. Md. T. Morshed, S. Rahman, S. Rahman, S. Jahan, M. Mukti, I. Taznin, M. H. R. Chawdhury and M. Rahmatullah, Jour. Adv. Nat. Appl. Sci. **7**(3), 260-263 **(**2013).
7. M. Liu, Y. Zhang, H. Zhang, L. Wan, H. Qin, Int. J. Biol. Macromol. **91,** 1170-6 (2016).
8. T. Ghosh, T. K. Maity, P. Sengupta, D. K. Dash and A. Bose. Iranian Jour. Pharm. Res. **7**(1), 61-68 (2008).
9. S. P. Baral and A. Alaknanda, Int. J. Pharm. Res. BioSci, (2014).
10. G. M. Walker, *Yeast Physiology and Biotechnology* (John Wiley & Sons, Chichester, 1998), pp. 362.
11. M. A. Hisamatsu, *Workshop Japan: JICA Short-term Expert* (JICA, Japan,1995).
12. M. F. Chaplin and J. F. Kennedy. *Carbohydrate analysis a practical approach. 2nd ed* (Oxford University Press, New York, 1994), pp. 73-94.

13. R. A. Copeland, *Method for Protein Quantitation In Method for Protein Analysis* (Springer, Boston, 1994), pp. 39-58.
14. M. Misra and U. Aiman, Indian J. Pharm. **44**(4), 538–539. (2012).
15. P. Molyneux, Jour. Sci. Tech. **26**(2), 211-219. (2004).
16. D. Tjokrokusumo, *Food mushroom diversity based on beta-glucan content and its health benefits* (Biodiversity, 2015), pp. 1520-1523.
17. M. Matheka, K. Duncan and Morris, Model. Pharm. (2012).
18. D. M. Aragao, L. Guarizo, J. Lani, J. C. de Costa and R. M. Garcia, E. Scio, J. Ethnopharm. **(3)**, 629-33 (2010).
19. O. M. Ighodaro, A. M. Adeosun and O. A. Akinloye, Med. (Kaunas), **53**(6), **365-374** (2017).
20. P. R. Hussain, S. A. Rather, and P. P. Surakdar, Rad. Phy. Chem. **144,** 218-230. (2018).
21. D. Fithriani, S. A. Susiana and S. Rini, J. Mar. Fish. Prod. Dev. (2015).