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Original Research Article

Anti-diabetic role of edible mushroom *Pleurotus sajor-caju*: Cultivated on different types of bed materials

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The cultivation of mushroom on different non-conventional bed may have some nutritional and physiological benefit with an additional solution of environmental remediation. In the present study *Pleurotus sajor-caju* has been cultivated on three different bed materials *i.e*., Paddy straw (PS), Banana Leaf-base (BL) and Sugarcane trash (SC). In the present study some physiochemical characters and antidiabetic role of cultivated mushrooms has been studied. The Insulin receptor affinity of the phytocomponents of *Pleurotus sajor-caju* identified in the literature also have been studied by Autodock Vina. Results show that the moisture content, water solubility, water absorption and swelling capacity has been found highest in SC mushroom compare to PS and BL mushroom. The elevated FBS in STZ-induced diabetic rats was recovered after dietary supplementation of mushroom cultivated on each bed materials. Interestingly, the recovery of elevated FBS was faster in rats which were treated with BL mushroom compared to that of other two groups. The affinity of phytocomponents ($C_{22}H_{34}O_4$, $C_{14}H_{17}O_9N$, $C_{16}H_{32}O_2$, $C_{17}H_{34}O$, $C_{18}H_{32}O_2$) towards Insulin receptors (pdbID: 1CE6 and 1gag) shows a good affinity value, that supports the probable antidiabetic role of *Pleurotus sajor-caju.* Therefore, it can be concluded that the more effective antidiabetic role of *Pleurotus sajor-caju* may obtained when cultivated on Banana Leaf-base.

Keywords: Edible mushroom, paddy straw, sugarcane trash, banana leaf base, diabetes

INTRODUCTION

Edible mushrooms are important sources of food with its test, flavor, nutritional value and easy digestibility. According to Aremu et al. (2009), the protein content of fresh mushroom is heights among all vegetables and fruits. On dry-weight basis, mushrooms are similar with respect to dried-yeast and superior to dried peas and beans (Aremu et al., 2009). There are evidences that the dried mature fruit bodies of mushroom may be used as nutraceuticals or dietary supplements. The physiological benefit of these dried forms has been described by Wasser 2014. Besides the supplementary use, the diet based beneficial effects of mushroom has been described by Sun and Niu (2020). The risk of gestational hypertension, preeclampsia, gestational weight gain and diabetes was low after consumption of mushroom as diet (Sun and Niu, 2020). The biological importance of different species of mushroom has been described by Venturella et al. (2021). There are evidences that different species of edible mushrooms has different physiological benefits with different nutrient content (Wani et al., 2010). The beneficial effect of mushroom proteins, carbohydrates, fats, vitamins and minerals on human health is the latest pharmacological research interest.

The mushroom cultivation is one of the profitable occupations due to its balanced recurring and non-recurring cost. In West Bengal, the *Pleurotus sajor-caju* is the most popular cultivated mushroom. The available paddy straw as the ingredients of the bed materials enhances the interest of cultivation of *Pleurotus sajor-caju*. The proper use of paddy straw as a conventional bed material is the approaches towards environmental remediation. But now a day the use of paddy cutting machine during the final stage of cultivation, the paddy straw are available as small cut pieces which are hard to carry and store. Therefore, it is pertinent to investigate the effects of other ingredients as bed materials which may be the alternative of the conventional paddy straw bed. In the present study, the sugarcane trash and banana leaf-base has been selected as bed materials for mushroom cultivation. The sugarcane trash and banana leaf-base have the silent features to be a representative of bed materials of mushroom cultivation. On the other hand, the use of these materials will be beneficial for environmental remediation. In the present study, the mushrooms cultivated on Paddy straw, Sugarcane trash and Banana-leaf base has been used to find some physical and physiochemical properties of mushroom with their antidiabetic role on Streptozotocin (STZ)-induced experimental diabetic rats.

Insulin plays a key role behind the glucose homeostasis and Diabetes Mellitus is the disease caused by the imbalance of this glucose homeostasis. The mechanism of insulin receptor activation by legend is important in this context. Therefore, the binding affinity of any bioactive molecule to the Insulin receptors may be the important parameter to find the role of that bioactive compounds towards glucose homeostasis. Some phytocomponents of *Pleurotus sajor-caju*is have been found in the literature (Kandasamy et al., 2020). In the present study, the affinity of those phytocomponents towards Insulin receptors has been studied by using Autodock Vina, the bioinformatics software for computational docking.

MATERIALS AND METHODS

Experimental Animals and Design of Experiment

Forty-eight (48) rats weighing 150-160 gm were equally divided into eight groups. According to the Institutional Animal Ethical Committee (Dean/2020/EC/1862) all the animals were kept into polypropylene animal cage in a standard laboratory condition with 12:12 hr light dark cycle. On the basis of the experimental design, the diet was prepared for each group. All the rats were allowed to drink water *ad libitum*. Eight groups are as follows: C, PSC, SCC, BLC, DC, PS, SC and BL. Control rats (C): Rats were treated with standard rats feed with no experimental manipulation. Paddy straw mushroom treated control (PSC): Rats were treated with mushroom (cultivated on paddy straw) without STZ administration. Sugarcane trash mushroom treated control (SCC): Rats were treated with mushroom (cultivated on sugarcane trash) without STZ administration. Banana-Leaf base mushroom treated control (PSC): Rats were treated with mushroom (cultivated on banana leaf base) without STZ administration. Diabetic Control (DC): Experimental STZ induced diabetic rats treated with normal rat feed. Paddy Straw mushroom treated diabetic rats (PS): STZ induced diabetic rats treated with dried mushroom (cultivated on paddy straw) as principal protein source. Sugarcane Trash mushroom treated diabetic rats (SC): STZ induced diabetic rats treated with dried mushroom (cultivated on sugarcane trash) as principal protein source. Banana Leaf-base mushroom treated diabetic rats (BL): STZ induced diabetic rats treated with dried mushroom (cultivated on banana leaf base) as principal protein source. Body weight and Fasting Blood Sugar (FBS) were measured in different time point in each group of rats. Treatment of dried mushroom through diet in PS, SC and BL rats was started after two weeks of the administration of STZ and continued up to 8 weeks. The mushroom treatment through diet in PSC, SCC and BLC rats were started at the same time.

Preparation of bed for mushroom cultivation

Paddy straw, sugarcane trash and banana leaf-base (mid rib) were collected from local market for the preparation of bed. For the preparation of paddy straw bed, the straw were cut into small pieces (approximately 1 to 1.5 inch) and steeped into water for 24 hrs containing 5gm/L bavistin. After 24 hr. the water was drained and the pieces were spread in shade for 01 hr. After that the bed was prepared by that shade dried straw. For the preparation of banana leaf base bed, the mid rib of the banana leaves were sliced into small pieces (approximately 1 to 1.5 inch) and exposed in sunlight for a whole day. The dried pieces of leaves were steeped into water for 24 hrs containing 5gm/L bavistin. After 24 hr. the water was drained and the pieces were spread in shade for 01 hr. After that the bed was prepared by those pieces of banana leaf-base. For the preparation of sugarcane trash bed, the hard cover of the sugarcane trash was removed and then cut into small pieces (approximately 1 to 1.5 inch). The small pieces were dried under sunlight for a whole day. Then, the pieces were boiled in water for 01 hr. and dried under shade for a whole day. These stapeses of boiling and shade drying were repeated for 5 days. The bavistin (5gm/L) was used at last the step of the boiling. After 5 days, the shade dried pieces were used for the preparation of bed.

Preparation of Mushroom Dust

In the Institutional Mushroom Cultivation Training Centre of Prabhat Kumar College, the mushroom (*Pleurotus sajor-caju)* was cultivated on three different bed materials i.e., Paddy Straw, Sugarcane trash and Banana Leaf-base. After cultivation, the mature fruit bodies of mushroom were collected and dried by the hot air oven at 50˚C - 55˚C for 48 hr. After that, the dried mushrooms were transferred into dust by the help of mixture grinder. The dust mushroom was kept in desiccator for future use.

Measurement of Physiochemical characters

The moisture content of the mushroom dust was determined according to (AOAC, 1995). The sample was dried at 105°C and the moisture content was calculated by the difference between the weight of dust before and after drying. Water solubility, water absorption and swelling capacity was calculated according to AOAC (1995).

Induction of STZ-induced diabetes in rats

STZ was dissolved in 0.1 M Citrate Buffer (pH 4.5) and injected intraperitonially (60 mg/Kg body weight) to the overnight fasted rats. To prevent the STZ induced hypoglycaemic shock, the rats were treated with 6% dextrose solution after 6 hr of STZ administration. After 72 hr of STZ administration, the experimental diabetes was confirmed by the fasting blood glucose level. The blood from tail vein of the overnight fasting rats was collected and the blood sugar level was tested by the Blood Sugar measurement Kit (Glucometer) by glucose-peroxide method using strips. The treatment of dried mushroom on diabetic rats was started after 2 weeks of STZ

Table 1. Moisture content, Water solubility, Water absorption and Swelling capacity of mushrooms cultivate on different bed materials.

Mushroom	Biological Efficacy	Moisture content	Water solubility	Water absorption	Swelling capacity
PS	83.16±0.795***	80.81±0.242	30%	54.5%	9.71%
BL	75.83±0.544	86.80±0.244	43%	63.5%	13.6%
SC	71.50±0.621	91.02±1.608**	50%	71.5%	14.0%

Values are presented as Mean ± SEM. * p<0.01, *** p<0.001

administration.

Treatment of rats with dried mushroom

To find the antidiabetic effect of mushroom, the dried mushroom was used in the present study. The dried whole mushroom dust has been used in the present study to identify the potentiality of these mushrooms as diet. The food of experimental groups of rats was prepared in such a way that the mushroom was the major source of protein (1gm/kg body weight) in the diet. For these experimental groups of rats, no other proteins have been used in the present study. The proximal composition (Protein, fat, carbohydrate and energy) of dried *Pleurotus sajor-caju* has been used to prepare the mushroom diet for the present experiments (Valverde et al., 2005).

Measurement of Receptor-legend affinity by Autodock Vina

Autodock Vina is one of the most widely used bioinformatics software for computational docking. In the present study, the affinity of some phytocomponents of mushroom (*Pleurotus sajor-caju:* reported in the literature by Kandasamy et al., 2019) towards Insulin receptors (pdbID: 1CE6 and 1ggg) was identified by Autodock Vina. The affinity value of the receptors was considered as percentage of affinity.

Statistical analysis

Values are expressed in Mean ±SEM. The One-way ANOVA followed by Dunnett Test was performed to compare the significant difference among three groups. The GraphPad Statistical Software was used to performed the tests.

RESULTS

Biological efficacy and some physiochemical properties (Moisture content, Water solubility, Water absorption and Swelling capacity) of mushroom cultivate on different bed materials.

The biological efficacy was found 83.16 ± 0.795 in PS mushroom, 75.83 ± 0.544 in BL mushroom and 71.50 ± 0.621 in SC mushroom. The biological efficacy was significantly higher [F=79.915, (2,15)] in PS mushroom than BL and SC mushroom. The moisture content of SC mushroom (91.02±1.608) is significantly higher [F=29.415, (2,15)] than PS and BL mushroom. The percentage of Water solubility (50%), Water absorption (71.5%) and Swelling capacity (14.0%) are also found highest in SC mushroom among three mushrooms (Table 1).

Effects of mushroom cultivated on different bed materials on fasting blood sugar level (mg/dL) in control and experimental rats.

The fasting blood sugar level were measure in different time points in different groups of rats. In C, PSC, SSC and BLC rats, the FBS level was not significantly altered in any time point of the present study. But in DC rats, the FBS level was significantly increased [F=17.862, (5, 30)] in different time point compared to the FBS of before STZ administration. In PS diabetic rats, the FBS level was significantly increased [F=66.910, (5, 30)] after 2 weeks of STZ administration and the elevated level was recovered after 8 week of the mushroom treatment cultivated on paddy straw. The significantly elevated [F=138.2, (5, 30)] FBS level in SC diabetic rats was recovered after 8weeks of mushroom treatment cultivated on Sugarcane trash. But in BL diabetic rats, the significantly elevated [F=193.98, (5, 30)] FBS level was recovered after 6week of mushroom treatment cultivated on Banana leaf base (Table 2).

Effects of mushroom cultivated on different bed materials on body weight in control and experimental rats.

In C, PSC, SSC and BLC rats the body weight was significantly increased [F=7.374, (5, 30)] with the progression of time in the present study. After administration of STZ, the body weight of rats was significantly decreased [F=24.742, (5, 30)] compared to the before STZ administration body weight. The recovery of decreased body weight was found in PS, SC and BL rats, after treatment of mushroom. In PS rats, the body weight was not significantly decreased [F=8.682, (5, 30)] compared to before STZ administration body weight after 4weeks of mushroom treatment and the same result was found after 6 weeks of treatment in SC [F=14.305, (5, 30)] rats. But in BL rats the decreased body weight was significantly recovered [F=14.141, (5, 30)] after 4weeks of treatment (Table 3).

Affinity of some phytocomponents of mushroom (identified in literature) towards Insulin receptors measured in Autodock Vina.

The values of the ligand receptor affinity are expressed in the Table 4. Results show that the $C_{14}H_{17}O_9N$ ligand has the maximum affinity towards the receptors 1CE6 (-7.7) and 1gag (-6.8). The lowest affinity has been found in $C_{17}H_{34}O$ towards the receptor 1CE6 (-5.1) and 1gag (-3.8).

Table 2. Effects of dried mushroom as principal protein source on fasting blood sugar (mg/dL) in mushroom treated control and STZ-induced diabetic rats.

Groups	FBS Initial / before STZ administration)	FBS (After 2 weeks / 2 weeks of STZ administration)	Fasting Blood Sugar after treatment with mushroom			
			2nd Week	4th Week	6th Week	8th Week
C	89.8±2.464	87.8±2.409	93.3±1.826	90.3±2.440	90.6±1.178	92.6±1.751
PSC	92.3±2.521	90.3±1.751	98.6±1.287	97.3±2.002	91.5±2.496	84.5±2.285
SCC	94.3±3.128	95.00±2.932	87.6±2.787	85.6±2.370	86.0±1.036	85.3±1.826
BLC	91.5±3.161	91.16±2.820	86.0±2.024	83.1±1.765	75.3±1.632**	72.5±1.365**
DC	94.1±1.858	253.3±21.704**	275.5±26.755**	298.0±20.83**	307.83±20.464**	323.33±19.020**
PS	86.3±3.435	244.3±13.717**	208.3±9.122**	177.5±6.566**	130.8±4.301**	103.0±2.105
SC	91.1±3.313	272.3±9.571**	237.6±4.648**	174.0±8.221**	133.1±5.531**	107.8±3.082
BL	92.0±3.110	291.3±10.826**	201.1±5.408**	126.3±4.373**	103.0±3.931	90.8±3.189

Values are presented as Mean ± SEM. * p<0.05, ** p<0.01

Table 3. Effects of dried mushroom as principal protein source on body weight (gm) in mushroom treated control and STZ-induced diabetic rats.

Groups	Body Weight (initial / before STZ administration)	Body Weight (After 2 weeks / 2 weeks of STZ administration)	Body Weight after treatment with mushroom			
			2nd Week	4th Week	6th Week	8th Week
C	153.5±2.455	160.5±3.579	166.3±3.671	170.6±4.054*	175.6±3.970**	180.5±4.005**
PSC	154.1±2.772	158.3±3.160	167.5±3.020*	172.0±3.227**	176.3±3.503**	182.8±2.597**
SCC	153.1±3.114	162.3±2.574	168.1±3.252**	172.3±2.951**	178.5±2.665**	184.6±3.771**
BLC	152.1±2.698	164.0±3.428*	169.83±2.395**	173.0±2.681**	182.1±3.071**	190.1±3.343**
DC	154.5±2.210	142.1±2.047	133.8±1.803**	124.8±3.48**	116.5±4.634**	107.0±5.235**
PS	157.8±2.324	143.6±2.548**	136.3±2.508**	142.6±2.870**	148.3±3.052	154.5±7.176
SC	148.0±2.630	138.6±2.353**	130.1±1.876**	131.6±1.387**	137.6±1.287**	145.6±1.503
BL	152.5±1.654	143.3±2.521*	137.0±2.579**	144.1±1.822	152.3±2.468	160.3±2.147

Values are presented as Mean ± SEM. * p<0.05, ** p<0.01

Table 4. Affinity of some phytocomponents of mushroom (identified in literature) towards Insulin receptors measured in Autodock Vina.

Sl. No	Ligand Name	Affinity value of Receptor pdbID: 1CE6	Affinity value of Receptor pdbID :1gag
i	$C_{22}H_{34}O_4$	-5.7	-4.9
ii	$C_{14}H_{17}O_9N$	-7.7	-6.8
iii	$C_{16}H_{32}O_2$	-5.0	-4.5
iv	$C_{17}H_{34}O$	-5.1	-3.8
v	$C_{18}H_{32}O_2$	-5.2	-4.8

DISCUSSION

In the present study, the biological efficacy and some physiochemical properties like moisture content, water solubility, water absorption and swelling capacity of mushroom dust were measured. Though the biological efficacy was found higher in PS mushroom but the non-conventional bed of BL and SC shows the effective biological efficacy. Therefore, the banana leaf base and sugar cane trash may be used as bed materials for environmental remediation. On the other hand, the moisture content, water solubility, water absorption and swelling capacity was found higher in SC mushroom indicates the bed materials may have some impact on the physiochemical properties of mushroom.

The role of mushroom dust as protein source in diet has been observed in STZ induced diabetic rats. Moreover, the possible role of different bed materials on the antidiabetic effects of mushroom also has been studied. Results show that, in STZ-induced DC rats the continuous increase of FBS indicates the efficacy of STZ on experimental diabetic rats. The dietary treatment of mushroom (cultivated on different bed materials) shows positive recovery effects on elevated FBS in rats. In PS and SC rats, the elevated FBS was recovered after 8 weeks of treatment. But in BL rats, the recovery of elevated FBS was faster than other two groups of rats. The recovery was found after 6 weeks of treatment, indicates the potentiality of antidiabetic role of mushroom is height when it is cultivated on Banana leaf-base. According to Vu et al. (2018 and 2019), the different parts of banana tree are the potential source of phenolic compounds. Phenolic compounds are the most abundant antioxidants in the diet of humans. There are thousands of natural polyphenols in the plant kingdom (Vu et al., 2019). Polyphenols, such as flavonoids, phenolic acid, and stilbenes, are a large and heterogeneous group of phytochemicals in plant-based foods (Vu et al., 2018). Growing evidence indicates that various dietary polyphenols may influence blood glucose level and may also help to prevent the diabetes (Aryaeian et al., 2017; Asgar Md A, 2013). In the present study, the BL mushroom may contain the phenoloic compounds which were cultivated on banana leaf-base. The results of the faster recovery of the elevated FBS in BL rats may support this context. Moreover, the slight depression of the FBS (though not significant) in BLC rats also supports the present observation.

Body weight of the rats was measured in different time point to study the effects of mushroom on growth in different experimental rats. In C rats, the continuous significant increase of the body weight indicates the natural physiological growth of rats in the present study. The significant increase of body weight was also found in mushroom treated control rats (PSC, SCC and BLC). These results indicate that the only mushroom protein used in the present study is sufficient for the normal growth. The continuous significant decrease of the body weight in STZ-induced DC rats indicate the positive effects of STZ in the present study. The effect of mushroom, cultivated on Paddy straw (PS) shows a recovery of body weight after 6 weeks of treatment in STZ-induced diabetic rats. In case of mushroom cultivated on Sugarcane trash (SC), shows the recovery effect on body weight after 8th weeks of treatment in STZ-induced diabetic rats. More effective result of mushroom treatment on body weight recovery was found in rats those were treated with mushroom cultivated on Banana Leafe-base (BL). In case of BL rats, the body weight was recovered after 2 weeks of treatment. The recovery of the body weight in STZ-induced diabetic rats is the indicator of the antidiabetic potentiality of the mushrooms used in the present study. Results of the body weight recovery in the BL rats support this context.

To find the possible affinity of the phytocomponents present in mushroom with insulin receptors, the bioinformatics software has been used in the present study. Some of the phytocomponents of mushroom (*Pleurotus sajor-caju)* has been found in the literature (Kandasamy et al., 2019). The affinity of those compounds has been identified towards Insulin receptors (pdbID: 1CE 6 and 1 ggg) by Autodock Vina. The result of Autodock Vina, for Ligands (Compounds of mushroom) – Receptors (Insulin receptors) affinity shows a good range of affinity value. Results also indicates the different affinity value of same compounds with different receptors. However, the phytocomponents of mushroom may have some antidiabetic effects on the basis of bioinformatics approaches.

The role of Insulin Receptors in glucose homeostasis and diabetes is one of the recent interests in biotechnological research. The antidiabetic role of different phytocomponents acting through Insulin receptors is important in this context. In the present study the Insulin receptor affinity of some phytocomponents of the edible mushroom *Pleurotu ssajor-caju* has been studied by Autodock Vina Software, the Bioinformatics software for computational docking. Molecular docking is a tool to find the most favourable binding pose of a receptor molecule relative to another when a complex is formed (Prasad et al., 2020). The negative binding affinity or negative binding free energy indicates the strong possibilities of ligand receptor binding. The highest binding affinity of any compounds shows the least binding energy value (Kollur et al., 2021). Results of the present study shows the negative binding affinity for all the phytocomponents tested in the present study to the Insulin receptors. The Insulin receptors, Tyrosine Kinase Domain andMHC Class I H-2db Heavy Chain were used as receptors against the Bio-compounds of Mushroom as ligands. Therefore, it is clear that all the studied active Bio-compounds of the edible mushroom *Pleurotus sajor-caju* in the present study have binding affinity to the Insulin receptors. Among the 05 ($C_{22}H_{34}O_4$:10, 12- Docasadiyndioic acid; $C_{14}H_{17}O_9N$: Tetraacetyl-D-xylonic nitrile; $C_{16}H_{32}O_2$: N-Hexadecanoic acid; $C_{17}H_{34}O$: Hexadecanal, 2-methylo; $C_{18}H_{32}O_2$: 9, 12-octadecadienoic acid) phytocomponents, the Tetraacetyl-D-xylonic nitrile ($C_{14}H_{17}O_9N$) shows the highest binding affinity to the Insulin receptors 1CE6: Chain A, Protein (MHC Class I H-2db Heavy Chain). On the other hand, Hexadecanal, 2-methylo ($C_{17}H_{34}O$) shows the least binding affinity to 1 gag: Chain A, Insulin Receptor, Tyrosine Kinase Domain. However, from the *In-silico* Bioinformatics approach of the present study, it can be concluded that some Bio-active compounds of edible mushroom *Pleurotus sajor-caju* has the binding capacity to the Insulin receptors.

In the present study, the in-vitro antidiabetic role of *Pleurotus sajor-caju* on STZ induced diabetic rats has been studied to support the results of the *in-silico* study. The reduction of body weight after administration of STZ and elevation of FBS in rats confirms the diabetes in experimental rats. The effect of *Pleurotus sajor-caju* has been assessed through dietary treatment of dried mushroom as principal protein source. No other protein has been used in diet except the mushroom. After eight weeks of the beginning of mushroom treatment in STZ induced diabetic rats the FBS shows the normal value, indicates the antidiabetic role of the dietary treatment of dried mushroom. Slight decrease (not significantly decreased) of the FBS was noted in BLC rats after treatment and the amount of mushroom per day in diet will be the concern parameter behind the present observation. Therefore, the dose and duration of treatment of mushroom as diet is important for its biological activity. The serum Insulin level of treated rats is also important behind the role of any antidiabetic agents. According to Ramachandran et al. (2012), the serum Insulin was significantly decreased in STZ induced diabetic rats and after experimental treatment the decreased serum Insulin was found to be normal with normal blood sugar level. Though the serum Insulin level was not measured in the present study, but the positive affinity of the Bio active compounds of *Pleurotus sajor-caju* to the Insulin receptors may support the involvement of Insulin.

The effect of cooked button mushroom (100 gm/ day) as diet on gestational diabetes was observed by Sun and Neu. (2020). According to Khatun et al. (2007), the consumption of 50 gm of cooked mushroom (*Pleurotus osteatus*) thrice daily for 24 days may reduce the plasma glucose in individuals. Therefore, from the above evidences it can be stated that the *Pleurotus sp.* of edible mushroom may has the antidiabetic role in the form of fresh, cooked, or dried. The observations of the present study may support this perspective.

From the present study it can be concluded that the edible mushroom *Pleurotus sajor-caju* may be used as beneficial functional foods for diabetic mellitus and the affinity of some active Bio-compounds of *Pleurotus sajor-*

caju to Insulin receptors is important in this context. It can be also concluded that the bed materials of the mushroom cultivation may have some influencing factor behind the antidiabetic role of that mushroom. In the present study, the banana leaf-base is found to be the most potential bed material for mushroom cultivation.

Conflict of interests

The authors declare that they have no conflicting interests.

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