Research Article

Evaluation of *in vitro* antidiabetic effect and phytochemical screening of some wild mushroom extracts isolated in Basrah, Iraq

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ABSTRACT

Background: Diabetes mellitus is a cardiovascular risk associated with chronic hyperglycemia in the blood; it is considered a modern-day epidemic and is really known as global public health. The aim of the present study was to assess the antidiabetic activity of some wild mushroom extracts which grown in Basrah Province. **Materials and Methods:** Ethanolic extracts were prepared from mushrooms fruiting bodies and their phytochemical analysis was determined. However, the ability of mushroom extracts to reduce the level of blood glucose *in vitro* was evaluated through α-amylase inhibition assay and glucose diffusion method, as well as, the detection of crude extracts cytotoxicity was examined. **Results:** The results were revealed the isolation of 12 species of mushrooms due to five genera including *Agaricus* spp. (four species), *Coprinus* spp. (two species), *Panaeolus* spp. (two species), *Psathyrella* spp. (three species), and *Scleroderma* sp., while the phytochemical assay was showed the dominance of polysaccharides, saponins, and tannins. Since the highest effect of α-amylase inhibition was seen at 500 µg for *Agaricus* sp. 1 and *Coprinus* sp. extracts (91%) followed by *Scleroderma* sp. extract (90%). **Conclusion:** The value of index for impaired glucose distribution glucose diffusion retardation index was reached after 3 h for all mushroom extracts, whereas the result was showed increasing value with time and concentration. Mushroom extracts were not revealed cytotoxic effect at doses of 5, 10, 50, 100, and 200 µg except *Psathyrella* sp. 2 and *Panaeolus* sp. 2 extracts that seen cytotoxicity at doses of 200 and 100 µg, respectively.

KEY WORDS: Antidiabetic, Cytotoxicity, Ethanolic extracts, Mushrooms

INTRODUCTION

Diabetes mellitus is a cardiovascular risk associated with chronic hyperglycemia in the blood; it is considered a modern-day epidemic and is really known as global public health. However, the number of diabetic people is expected to increase from present estimate of 150 million to 230 million in 2025.[1,2] Hyperglycemia increases the production of free radicals that cannot be inhibited by the body. This will thus cause oxidative stress in the cell and will decrease the enzymatic antioxidant defenses resulting in diabetes.[3] Antioxidant supplementation is, therefore, essential as it helps in inhibition the harmful action of free radicals on insulin secretion, thus balancing the glycemic index.[4]

The major mode of controlling diabetes can be achieved by diet, exercise, and insulin replacement therapy and/or by difference oral hypoglycemic drugs. However, treatment with sulfonylureas and biguanides is associated with side effects and fails to alter the course of diabetic complications significantly.[5,6] In modern medical system, managing diabetes without side effects is still a challenge. Nowadays, herbal medicines are highly recommended for the treatment of diabetes in spite of the therapeutic option. Since antiquity people have used different medical herbs as antidiabetic remedy, because it is considered to be less toxic and induce fewer side effects than synthetic ones.[7] Mushrooms are edible fungi which have been used as antioxidants since ancient time and thus prevent from certain metabolic disease like diabetes. Mushrooms are nutritive and are richer in protein than cereals, pulses, fruits, and vegetables on dry weight.[8,9]

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MATERIALS AND METHODS

Samples Collection and Processing

Fresh fruiting bodies of wild mushrooms were collected from various locations in Basrah Province

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in South Iraq, from December 2017 to April 2018. Standard methods of collection, preservation, and identification were followed according to several references.[10-12] Fresh samples were dried in the laboratory and powdered to prepare for extraction.

Preparation of Ethanolic Extracts

Fifty grams of dried mushrooms were extracted by boiling and stirring with 500 ml ethanol at 150 rpm for 20 min, then filtered through Whitman No. 1 filter paper. Ethanol extract was subjected with rotary evaporated at 45°C for 2 h. The concentrated extract was dried moreover and stored in a sterile dark bottle at −20°C until used.[13]

Chemical Screening

The extracts were subjected to different chemical tests to detect their major constituents. The tests were performed as follows:

- Polysaccharide determination: The quantification of polysaccharides was accomplished using the method described by Masuko *et al.*[14] [Figure 1a].
- Bradford assay (proteins test): Quantification of protein was carried out by protein-dye binding method[15] [Figure 1b]. The other chemical screenings were performed to identify chemicals in the ethanolic extracts of mushroom that used in the study according to Thilagavathi *et al*.[16]

α-amylase Inhibitory Assay

The inhibition assay was performed using the chromogenic DNSA method as described by Parimelazhagan *et al*.[17] and Paul and Banerjee.[18] It was used to measure enzyme activity by measuring the amount of reducing sugars formed. Stock solutions of all extracts (1000 ppm) were prepared by adding 1 g of each extract to 1 L of phosphate buffer saline (PBS); then, serial dilution of extracts was composed with PBS (100 µg, 200 µg, 300 µg, and 400 µg). After that, 100 µl of α-amylase solution (0.001 g of α-amylase was dissolved in 100 ml of 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride) were added to each test tube, 100 µl of different concentrations of tested samples were added (100 µg, 200 µg, 300 µg, and 400 µg) in each tested tube separately. All tubes were incubated at 25°C for 10 min. A 100 µl of 1% starch solution were added to each tube, all tubes were incubated at 37°C for 10 min; then, 200 µl of 3,5-dinitrosalicylic acid (DNSA) reagent was added and incubated in hot water bath (85°C). After 5 min, the color of mixture was changed to orange-red and removed from water bath. All samples were cooled at room temperature, then diluted with 2 ml distilled water. Absorbance was taken at 540 nm. A control was prepared using the same procedure replacing the extract with distilled water while blank was performed by replacing enzyme with buffer. The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\% \text{ Inhibition} = \{Abs_{control} - Abs_{extracts}/Abs_{control}\} \times 100.$$

Where, $Abs_{control}$ that absorbance of control
$Abs_{extracts}$ that absorbance of extracts.

Glucose Diffusion Method

The potential of mushroom extracts to inhibit glucose diffusion into the external solution was investigated at set time intervals.[19] Four different concentrations of crude extract (200, 150, 100, and 50 µg/ml) were prepared. A 1 ml of extract was placed in a dialysis membrane and 1 ml of 0.22 mM glucose in 0.15 M of NaCl was added. The dialysis membrane was tight at both ends and immersed in a beaker containing 40 ml 0.15 M of NaCl and 10 ml of distilled water, for control, 1 ml of 0.22 mM glucose in 0.15 M of NaCl was added in dialysis membrane bag along with 1 ml of distilled water and immersed in a beaker (40 ml 0.15 M of NaCl + 10 ml distilled water). The beakers were kept at room temperature. The glucose movement from internal solution to external solution (beaker solution) was measured every ½ h by glucose oxidase kit method. Three replicates were done for every ½ h for 3 h. A standard curve was drawn using different glucose concentrations. The glucose diffusion retardation index (GDRI) was calculated using the following formula:

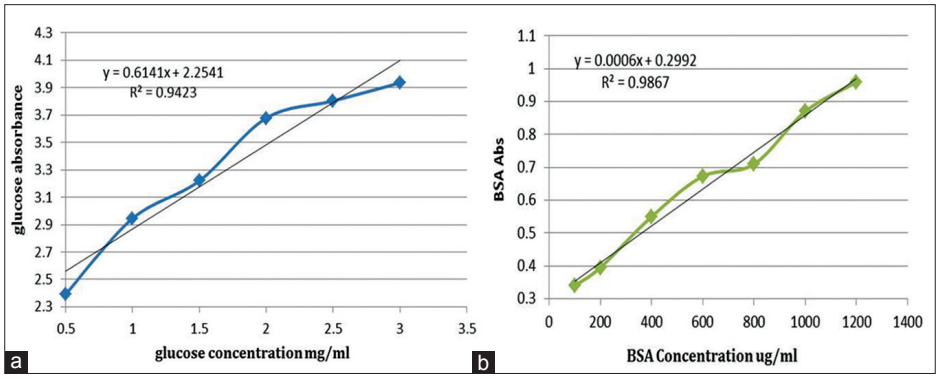

Figure 1: Stander carve of (a) glucose and (b) bovine serum albumin

GDRI = 100 – ([Glucose content (mg/ml) in external solution in the presence of extract/glucose content (mg/ml) in external solution in the absence of extract] * 100).

Cytotoxicity Assay

The method was described by Xian-guo and Ursula[20] who were employed to study cellular toxicity. A 10-fold serial dilution of mushroom extracts was made in phosphate-buffered saline (400, 350, 300, 250, 200, 150, 100, 50, 25, and 10 µg/ml). A total volume of 0.8 ml for each dilution was placed in an Eppendorf tube. A negative control tube (containing saline only) and a positive control tube (containing tap water). Fresh blood were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37°C for 30 min, all tubes were centrifuged for 5 min and then observed for hemolysis of erythrocytes. Complete hemolysis was seen by a clear red solution without any deposit of erythrocytes. Hemolysis was also checked microscopically for the presence or absence of intact red blood cells.

RESULTS

Isolation and Identification

During the study periods, 12 macrofungi species were reported due to five genera that included *Agaricus* spp. (four species), *Coprinus* spp. (two species), *Panaeolus* spp. (two species), *Psathyrella* spp. (three species), and *Scleroderma* sp.

Table 1: Polysaccharide and protein content (g/L) and pH of extracts

Extracts	Polysaccharide	Protein	pH
Agaricus sp. 1	5.40	0.63	6.0
Agaricus sp. 2	8.33	--------	6.0
Agaricus sp. 3	2.00.	-------	6.0
Agaricus sp. 4	6.16	1.52	6.0
Coprinus sp.	5. 00	--------	6.0
Coprinus sterquilinus	0.84	--------	6.0
Panaeolus sp. 1	12.00	--------	6.0
Panaeolus sp. 2	3.25	---------	6.0
Psathyrella sp. 1	2.70	-------	6.0
Psathyrella sp. 2	3.00	-------	5.4
Psathyrella sp. 3	4.20	--------	6.7
Scleroderma sp.	12.94	0.28	5.0

Chemical Assays for Mushroom Extracts

The primary detection of chemical compounds which found in mushroom extracts is shown in Tables 1 and 2. The result of Table 1 was showed that specific chemical tests were done to find polysaccharide and protein content of all extracts.

In vitro Experiments

Antidiabetic activity of α-amylase inhibition

The effect of extracts on α-amylase activity which presented in terms of enzyme inhibitory activity (%) was showed increasing inhibition of α-amylase that noted at 200, 300, 400, and 500 µg of all extracts [Figures 2-6], while the extracts of *Agaricus* spp. were showed that maximum inhibition of α-amylase followed by *Coprinus* spp. and *Scleroderma* sp. at 500 µg for all [Figures 2 and 3]. The outcomes of the present study were suggested that the extract exhibited

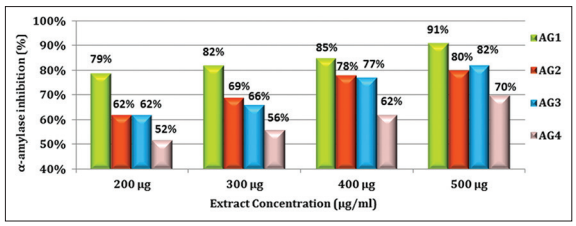

Figure 2: α-amylase inhibition assay of *Agaricus* spp. extracts: *Agaricus* sp. 1 (AG1), *Agaricus* sp. 2 (AG2), *Agaricus* sp. 3 (AG3), and *Agaricus* sp. 4 (AG4)

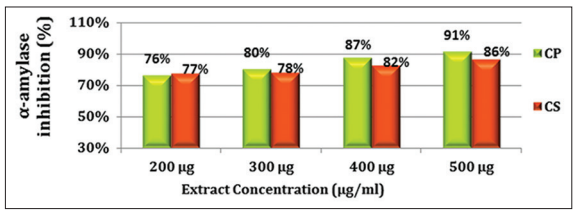

Figure 3: α-amylase inhibition assay of *Coprinus* spp. extracts: *Coprinus* sp. (CP) and *Coprinus sterquilinus* (CS)

Table 2: Chemical composition of extracts of mushrooms

Extracts	Phytochemical analysis								
	Flavonoid	Phenol	Alkaloid	Terpenoid	Tannin	Sterols	Saponin	Coumarin	Glycosides
Agaricus sp. 1	+	+	+	–	+		+	–	+
Agaricus sp. 2	+	+	+	–	+	–	+	–	+
Agaricus sp. 3	+	+	+	–	+	+	+	–	+
Agaricus sp. 4	+	+	+	–	+	–	+	–	+
Coprinus sp.	+	+	+	+	+	+	+	–	+
Coprinus sterquilinus	+	+	+	–	+	–	+	–	
Panaeolus sp. 1	+	+	+	–	+	–	+	–	+
Panaeolus sp. 2	+	+	+	+	+	–	+	–	+
Psathyrella sp. 1	+	+	+	+	+	+	+	–	+
Psathyrella sp. 2	+	+	+	+	+	–	+	–	+
Psathyrella sp. 3	+	+	+	–	+	–	+	–	+
Scleroderma sp.	+	+	+	–	+	–	+	–	+

+: Present, –: Not detected

Table 3: Glucose dialysis retardation index values for mushroom extracts

Extracts	50 µg			100 µg			150 µg			200 µg		
	GDRI after 1 h	GDRI after 2 h	GDRI after 3 h	GDRI after 1 h	GDRI after 2 h	GDRI after 3 h	GDRI after 1 h	GDRI after 2 h	GDRI after 3 h	GDRI after 1 h	GDRI after 2 h	GDRI after 3 h
AG1	26.94	32.94	39.65	30.73	35.72	40.94	37.83	38.96	42.52	40.31	43.21	45.84
AG2	23.85	30.64	33.93	27.68	30.98	34.06	28.32	33.43	34.74	28.88	34.01	34.95
AG3	18.98	22.91	28.86	20.76	27.31	30.23	23.74	28.94	30.86	26.85	30.85	31.54
AG4	19.27	20.72	25.82	20.04	21.86	27.03	20.98	25.75	29.66	22.84	26.65	30.04
CP	22.92	27.85	30.54	25.83	32.43	34.65	25.97	32.88	35.72	26.05	33.76	36.65
CS	23.34	30.65	35.89	28.43	33.66	38.65	29.76	33.97	39.06	30.64	35.95	39.83
PC1	25.32	31.93	32.45	25.98	32.93	35.42	27.84	34.73	35.89	29.05	35.32	36.11
PC2	24.12	28.87	30.44	26.75	29.03	32.62	27.85	30.43	34.31	29.65	32.73	34.92
PC3	23.76	25.55	28.84	24.96	26.32	30.02	26.44	27.56	32.18	26.92	28.74	33.12
PP1	17.12	21.54	26.75	20.21	23.82	29.43	22.85	25.97	30.98	24.74	28.66	31.07
PP2	25.65	28.85	30.21	26.87	28.99	31.15	27.42	31.88	33.05	28.32	32.07	33.86
SF	25.91	33.31	37.21	29.94	35.85	37.21	29.66	34.93	39.67	30.32	42.54	44.96

The mean of significant difference at the level 0.05. **Agaricus* sp. 1 (AG1), *Agaricus* sp. 2 (AG2), *Agaricus* sp. 3 (AG3), *Agaricus* sp. 4 (AG4), *Panaeolus* sp. 1 (PP1), *Panaeolus* sp. 2 (PP2), *Scleroderma* sp. (SF), *Coprinus* sp. (CP), *Coprinus sterquilinus* (CS), *Psathyrella* sp. 1 (PC1). *Psathyrella* sp. 2 (PC2), and *Psathyrella* sp. 3 (PC3)

significant ($P < 0.05$) inhibition of α-amylase enzyme compared with control (in the absorbance of 0.2050). The results were reflected the hypoglycemic activity of all mushroom extracts.

Effect of mushroom extracts on GDRI

Effects of mushroom extracts on GDRI with respect to time are reported in Table 3. GDRI maximum value was reached after 3 h for all mushroom extracts, while the values were increased with time and concentration. Based on above results, mushroom extracts of different concentration were showed high retardation index with indication of their hypoglycemic effect. At 3 h and 200 µg, the GDRI of all mushroom extracts was seen a significant difference ($P < 0.05$) higher than that of control. Similar trend was observed at 1 h and 2 h of incubation. The extract of *Agaricus* sp. 1 (AG1) was showed that higher GDRI values at 3 h, whereas the lower GDRI values at 3 h were observed of *Agaricus* sp. 4 extract (AG4). Depending on these results, all mushroom extracts were revealed different values of retardation index and possibly will help in hypoglycemic treatment.

Cytotoxicity Test

Different extracts of mushroom were not showed toxicity at a dose of 5, 10, 50, 100, and 200 µg except *Psathyrella* sp. 2 and *Panaeolus* sp. 2 extract that revealed cytotoxic effect at a dose of 200 µg and 100 µg, respectively [Figure 7 and Table 4].

DISCUSSION

The present study was revealed to the isolation of different species of mushrooms that indicated to the diversity of macrofungi occurrence in Iraq habitat, especially in Basrah in spite of extreme environmental conditions that included *Agaricus* spp. (four species), *Coprinus* spp. (two species), *Panaeolus* spp. (two species), *Psathyrella* spp. (three species), and *Scleroderma* spp. (one species). The most common factors that limit the growth of mushrooms are temperatures and rain seasons. When the year is a rainy season with abundant rain and frequent humidity, it leads to the growth of many types of wild mushrooms, especially species belong to the genus *Agaricus* spp. The *Panaeolus* spp. and *Psathyrella* spp. prefer growth and development under warm and moderate humidity conditions of more than 30°C. Therefore, the highest percentage of species that collected during the winter was due to *Agaricus* spp.; however, the species were collected in the non-rainy seasons belong to *Panaeolus* spp. and *Psathyrella* spp. It is worth mentioning that the year 2018 in Iraq was little of rain and a short winter, which led to the scarcity of the presence samples and the annual product of *Agaricus* spp. land is very low in comparison with the previous years when heavy rainfall.

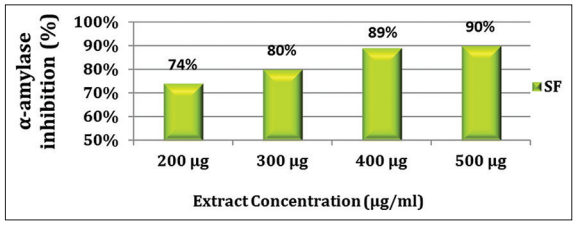

Figure 4: α-amylase inhibition assay of *Scleroderma* sp. extract (SF)

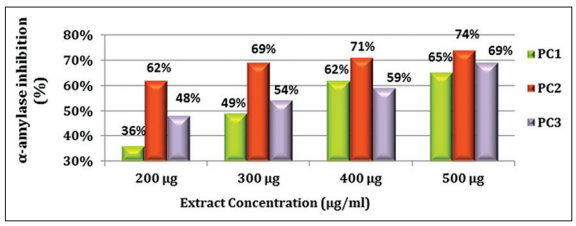

Figure 5: α-amylase inhibition assay of *Psathyrella* spp. extracts: *Psathyrella* sp. 1 (PC1), *Psathyrella* sp. 2 (PC2), and *Psathyrella* sp. 3 (PC3)

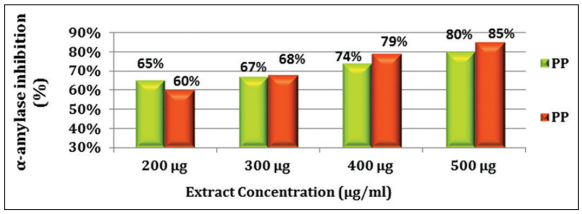

Figure 6: α-amylase inhibition assay of *Panaeolus* spp. extracts: *Panaeolus* sp. 1 (PP1) and *Panaeolus* sp. 2 (PP2)

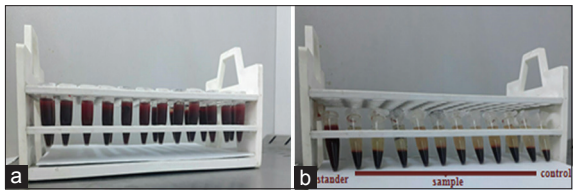

Figure 7: Cytotoxicity test, (a) Initial time, (b) After centrifuge

α-amylase enzyme is work as hydrolysis of α-1-4 glycosidic linkages from the non-reducing ends of polysaccharides (starch-amylase, amylopectine, and glycogen) to yield maltose units. This enzyme does not hydrolyze α-1-6-glycosidic linkages present in branch chain polysaccharide (amylopectin and glycogen). Alpha-amylase inhibitor is a proteinaceous substance, which binds with alpha-amylase enzyme and forms α-amylase enzyme-inhibitor complex and thereby inhibits α-amylase activity, which resulted in slow digestibility of starch and reduces rate of glucose absorption. Thus, postprandial rise in glucose is decreased.[21]

In the present study, α-amylase assay was revealed that mushroom extracts inhibited α-amylase activity at all concentrations of all extracts, this due to binding

Table 4: Cytotoxicity test of mushroom extracts

Extracts	5 µg	10 µg	50 µg	100 µg	150 µg	200 µg	250 µg	300 µg	350 µg	400 µg
AG1	−	−	−	−	−	−	−	−	−	−
AG2	−	−	−	−	−	−	−	−	−	−
AG3	−	−	−	−	−	−	−	−	−	−
AG4	−	−	−	−	−	−	−	−	−	−
CP	−	−	−	−	−	−	−	−	−	−
CS	−	−	−	−	−	−	−	−	−	−
PC1	−	−	−	−	−	−	−	−	−	−
PC2	−	−	−	−	−	+	+	+	+	+
PC3	−	−	−	−	−	−	−	−	−	−
PP1	−	−	−	−	−	−	−	−	−	−
PP2	−	−	−	+	+	+	+	+	+	+
SF	−	−	−	−	−	−	−	−	−	−

**Agaricus* sp. 1 (AG1), *Agaricus* sp. 2 (AG2), *Agaricus* sp. 3 (AG3), *Agaricus* sp. 4 (AG4), *Panaeolus* sp. 1 (PP1), *Panaeolus* spp. 2 (PP2), *Scleroderma* sp. (SF), *Coprinus* sp. (CP), *Coprinus sterquilinus* (CS), *Psathyrella* sp. 1 (PC1), *Psathyrella* sp. 2 (PC2), and *Psathyrella* sp. 3 (PC3)

between enzyme and specific compound found in mushroom extracts that form α-amylase enzyme-inhibitor complex, thus lowering postprandial glucose. The extracts of *Agaricus* sp. 1 and *Coprinus* sp. 1 were showed the maximum inhibition of α- amylase followed by *Scleroderma* sp., the inhibition activity may be as a result of presence active compound such as alkaloid, phenol, tannins, and some polysaccharide as β-glucan that plays the main role in lowering hyperglycemia, as well as other compounds such as lectin.[22] Based on the results obtained in a study of Kumar *et al*.[23] who were concluded that the extracted polysaccharides have significant antidiabetic activity. These results suggested that *A. bisporus* polysaccharides could be inhibiting α-amylase activity.

Glucose dialysis retardation index (GDRI) is a useful *in vitro* index to predict the effect of extracts on the delay in glucose absorption in the gastrointestinal tract.[24] Effects of mushroom extracts on GDRI with respect to time are reported in Table 3. GDRI maximum value was reached after 30 min for all extracts tested and values which rose as time increases for all extracts. The extract of *Agaricus* sp. 1 (AG1) was showed that higher GDRI values at 3 h, whereas the lower GDRI values at 3 h were observed of *Agaricus* sp. 4 extract (AG4). Thus, the results were indicated that the extracts could effectively bind to glucose even at low concentrations of glucose, thereby reducing the amount of accessible glucose in small intestinal. Based on above results, mushroom extracts showing high retardation index and possibly help in showing hypoglycemic effect. The present study was agreed with Silva *et al*.[25] who were showed that the activity of *A. campestris* extract was decreased glucose diffusion across the gastrointestinal.

Cytotoxicity results were not showed a toxic effect for most extracts indicating to possibility use as antidiabetic agent. The cause of the toxicity of the PC and PP extracts may be due to the toxic compounds which cannot be destroyed by heat during extraction because not all toxins are protein based. Some are small molecules that are compatible with some of the vital proteins in blood, causing them to hemolysis.[26]

It is apparent from the current study the biological activity of ethanolic extracts of mushrooms to inhibit α-amylase that indicated to hypoglycemic effect. In addition to delay sugar digestion and assist the obstruction of blood glucose level. The evidence to achievable for use these extracts as antidiabetic drugs.

CONCLUSION

The results pointed that mushroom extracts may be use in future (with more experimental studies) as antidiabetic agents because *in vitro* result elucidate the mechanism of extract action due to inhibition of α-amylase and abstraction of glucose diffusion, as well as, they have not any cytotoxicity effects *in vitro*.

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