Full Length Research Article

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Research Journal of Agricultural Sciences **14**(6): 2008–2011 (Nov-Dec 2023)
DOI: http://rjas.org/Article/Article/5728

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

In Vitro Antidiabetic and Antioxidant Activities of Ethanolic Extract of *Pleurotus florida* (Mont.) Singer Mushroom

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Received: 20 Sept 2023; Revised accepted: 08 Dec 2023; Published online: 29 Dec 2023

Abstract

Mushrooms are an excellent source of medicinal compounds. *Pleurotus florida* is both a medicinal and edible mushroom. Oyster mushrooms are the among the most prevalent culinary fungus. These mushrooms were high in proteins, carbs, and fibre, yet deficient in fat. These had a high mineral content as well. They are commonly used as a medicinal food because of its anticarcinogenic, anticholesterolemic, and antibacterial characteristics, as well as their preventive properties. This research delves at the anti-diabetic and antioxidant properties of the edible fungus *Pleurotus florida*, which is commercially grown in Trichy, Tamil Nadu. The antioxidant bioassay revealed that *Pleurotus florida* performed inhibitory activity. DPPH radical scavenging activity and ABTS tests were used to measure total antioxidant activity. The ethanolic extract inhibited activity at a concentration of 100µg/mL, which was 54.28 and 67.77% with conventional L-ascorbic acid. At a dosage of 100µg/mL, the mushroom extract also demonstrated considerable anti-diabetic (75 & 66%) efficacy via inhibition of -amylase and -glucosidase. These findings imply that *Pleurotus florida* could be investigated as a unique and potent natural antioxidant and anti-diabetic drug, as an alternative to currently available synthetic compounds. *Pleurotus florida* also boosts our immune system's ability to fight diseases caused by free radicals. As a result, it can be used as a dietary supplement alongside with other foods or as a medicine in and of itself.

***Key words*:** Mushroom, *Pleurotus florida*, Inhibitory, Anti-diabetic, Antioxidant, Ethanolic extract

According to the World Health Organization, cancer and diabetes mellitus are major causes of morbidity and mortality, affecting more than fifty percent of the population overall and one in five people over the age of 40 [3]. A global public health burden is created by the rising rates of adult-onset cancer and Type 2 diabetes around the world. Cells produce free radicals as part of their regular metabolic process [11]. Although cells have defences against these metabolic radicals, when they are overloaded, they become difficult to eliminate, which leads to oxidative stress [8]. Chronic and degenerative illnesses like cancer, autoimmune disorders, ageing, cataract, rheumatoid arthritis, neurological diseases are all greatly influenced by this process [7].

For countless years, people have utilized mushrooms as meal and nutritional supplements [6]. It is a crucial food in terms of nutrition, human health, and illness prevention. Major therapeutic effects of mushrooms include blood cholesterol lowering, anticancer, antibacterial, and antiviral activity. Because of their wide range of therapeutic properties, low toxicity, and lack of side effects [21], active compounds isolated from natural sources like algae, lichens, higher plants, and mushrooms have attracted a lot of attention in the biomedical field in recent decades [9]. Additionally, *Pleurotus* species have many medical benefits [24] and is a prevalent, lucrative mushroom found broadly in pleasant, subtropical, and tropical regions [10]. Additionally, Oyster mushrooms (*Pleurotus* spp.) can employ a variety of basal substrates more than any other mould [3].

According to [27], the *Pleurotus* sp. is a viable candidate for medicinal mushrooms because to its antibacterial, antiviral and haematological. These varieties of mushrooms contain biologically active substances like polysaccharides in addition to their distinct flavour, high nutritional value, and biological value [8]. In the current study, the anti-oxidant and anti-diabetic potential of crude extract extracted from the ethanolic extract of *Pleurotus florida* has been assessed [11]. In order to raise consumer knowledge of the positive effects of edible mushrooms, the investigation's goal is to examine the anti-diabetic and antioxidant properties of these Tamil Nadu-grown mushrooms.

MATERIALS AND METHODS

Formulation of Pleurotus florida extract

The *Pleurotus florida* edible mushroom was propagated in a clean space, and the fruit sections were scrubbed to eliminate debris before being air dried, crushed up, and extracted with ethanol in the ratio of 1:10 via a soaking method [23]. Following extraction, crude liquids were stored in a water bath (60 °C) to allow any excess solvents to evaporate [23]. The resulting extracts were collected and stored in a sealed container for future use. The resulting extract was used in further study.

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Citation: Sangeetha K, Senthilkumar G. 2023. In vitro antidiabetic and antioxidant activities of ethanolic extract of *Pleurotus florida* (Mont.) singer mushroom. *Res. Jr. Agril. Sci.* 14(6): 2008-2011.

Assessment of antidiabetic in vitro
α-amylase inhibition assay

The inhibitory activity of an amylase (10-100µg/mL) was examined using Wan *et al*. [28] approach, with a few modifications. In brief, the *Pleurotus florida* ethanolic extract and acarbose were mixed with 10 µL of α-amylase (pre-incubated in 20 mM sodium phosphate buffer, pH 6.7) and incubated at 37 °C for 5 minutes. The starch solution (0.2% w/v) was added to the reaction mixture to bring the total volume to 2 mL and incubated for 5 minutes at 37 °C. Following incubation, 1 mL of dinitrosalicylic acid reagent was added and the mixture was maintained in a boiling water bath [12]. After 5 minutes, the solution was allowed to cool before adding deionized water. At 540 nm, absorbance was measured, and α--amylase inhibition was estimated as follows:

$$\alpha\text{-amylase inhibition (\%)} = [Ac - As / Ac] \times 100$$

Where Ac and As denote the absorbance of control and extract, respectively.

The IC_{50} value was derived as the concentration of extract that inhibited α -amylase by 50%.

α -glucosidase inhibition assay

The α -glucosidase inhibitory activity was measured using modified Wan *et al*. (2013) approach. The *Pleurotus florida* extract and acarbose (10-100µg/mL) were combined with 75 µL of α -glucosidase (made in 0.1 M potassium phosphate buffer; pH 6.8) and incubated for 15 minutes at 37^0C. To commence the reaction, 20 µL of 5 mM para nitrophenyl-a-D-glucopyranoside (PNPG) produced in 0.1 M potassium phosphate buffer were added and incubated for 15 minutes. After incubation, the reaction was stopped by adding 80 µL of 0.2 M Na_2CO_3, and the absorbance at 405 nm was measured [13]. The inhibition of a-glucosidase was calculated as follows:

$$\alpha\text{ -glucosidase inhibition (\%)} = [Ac - As / Ac] \times 100$$

Where Ac and As denote the absorbance of control and extract, respectively.

The IC_{50} value was derived as the concentration of extract that inhibited α -glucosidase by 50%.

Assessment of antioxidant in vitro
DPPH radical scavenging activity

In order to determine the free radical scavenging activity of the crude extract, we used a slightly modified protocol of the very popular method of Brand-Williams *et al*. [5], which involves the reaction of chemical compounds in the test solution with DPPH radical (2,2'-diphenyl 1-picryl hydrazyl), which upon reaction with antioxidants, transfers the free radical to the electron accepting antioxidant compounds, leading to a loss of the intense pinkish purple colour of the DPPH radical. 1 mL of 0.1 mM DPPH radical solution was combined with 1 mL of the fruit body extracts of *Pleurotus florida* (prepared using different solvents) at varying concentrations (10-100 µg/ml). We prepared corresponding blank solutions and employed L-ascorbic acid (of equal concentrations as the test solutions, in the range of 10-100 µg/ml) as a positive control antioxidant known to rapidly scavenge free radicals [25]. We used a mixture of 1 mL ethanol with 1 mL DPPH as control. The disappearance of DPPH radical was monitored at 520 nm using a spectrophotometer, after incubation at room temperature in the dark. % Inhibition was calculated using the formula given below:

$$\text{Inhibition \%} = [Ac - As / Ac] \times 100$$

Where Ac represents the control's abs. and as represents the sample's abs.

ABTS assay

The decolorization of the ABTS radical cation is what gives *Pleurotus florida's* ethanolic fruit body extracts their antioxidant function. To a concentration of 7 mM, it was diluted with double-distilled water. The ABTS radical cation (ABTS•+) was produced through a 1:1 reaction with potassium persulfate (2.45 mM), and this reaction was allowed to proceed in the dark at room temperature for 12–16 hours. To bring the solution's optical density (OD) value to 0.7 (734 nm) after incubation, the solution (ABTS•+) was dissolved in double-distilled water. Additionally, 1 ml of freshly made solution (ABTS•+) was combined with 1 ml of *Pleurotus florida* fruit body extracts at various concentrations (10-100µg/mL). In order to determine the OD value, a spectrophotometer was used at 734 nm. Servings of ascorbic acid were the norm. The percentage of radical scavengers was calculated using the DPPH test.

RESULTS AND DISCUSSION

Assessment of antidiabetic in vitro
α-amylase and α- glucosidase inhibition assay

By the inhibition of - amylase, an enzyme that digests starch, and using Acarbose as a standard [14], the ethanolic *Pleurotus florida* extract was examined for anti-diabetic action. The results showed that the ethanol extract of *Pleurotus florida* at concentrations of 60 µg/mL and 100 µg/mL inhibited growth by 71.4% and 75%, respectively. Thus, the findings in (Table 1, Fig 1-2) demonstrate that *Pleurotus florida* extract is a potent diabetic drug [14]. Then α -glucosidase, an enzyme that breaks down starch as well, and using acarbose as a reference. The findings demonstrated that the growth was suppressed by 65% and 66%, respectively, by the methanol extract of *Pleurotus florida* at concentrations of 80 µg/mL and 100 µg/mL. The results in (Table 1, Fig 1-2) thus show that *Pleurotus florida* extract is an effective diabetes medication.

Table 1 α-amylase and α -glucosidase inhibition assay of ethanolic extract of *Pleurotus florida*

Concentration (µg/ml)	% of Inhibition		
	α -amylase inhibition	α -glucosidase inhibition	Standard (Acarbose)
10	61.4	53	61
20	64.2	55	72
40	64.3	59	84
60	71.4	62	92
80	67.9	65	100
100	75	66	100

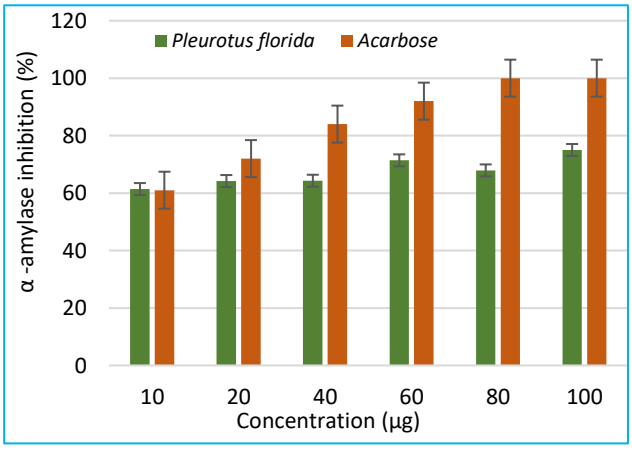

Fig 1 α-amylase inhibition assay of ethanolic extract of *Pleurotus florida*

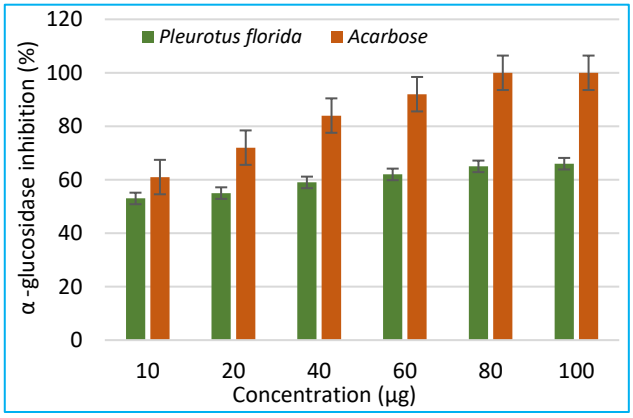

Fig 2 α- glucosidase inhibition assay of ethanolic extract of *Pleurotus florida*

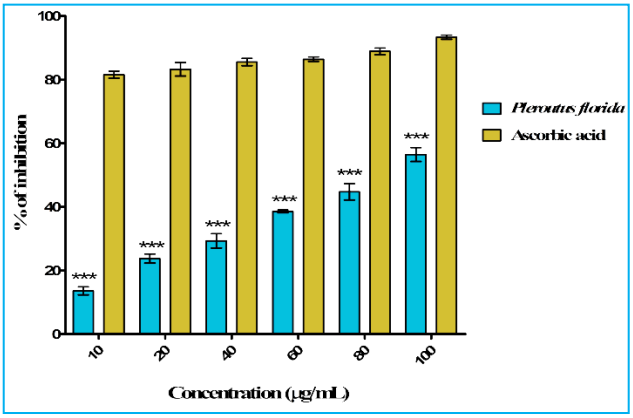

Fig 3 DPPH scavenging assay of ethanolic extract of *Pleurotus florida*

Assessment of antioxidant in vitro

DPPH scavenging and reducing power assays were used to test the antioxidant activity of the various *Pleurotus florida* crude extract concentrations. At 100 g/mL of extract, the *Pleurotus florida* ethanolic extract had the highest DPPH-scavenging activity (54.28%) (Fig 3, Table 2). The six *P. florida* extracts had a 54.28% reducing power at 100 µg/mL and a 0.51-0.97 reducing power at 500 µg/mL (Fig 4, Table 2).

Table 2 DPPH scavenging and ABTS inhibition assay of ethanolic extract of *Pleurotus florida*

Concentration (µg/ml)	% of inhibition			
	DPPH scavenging	Standard (Acarbose)	ABTS	Standard (Acarbose)
10	14.89	82.69	17.34	65.46
20	22.34	85.42	24.19	71.06
40	27.08	86.71	37.16	77.14
60	39.04	87.14	45.61	79.26
80	47.31	89.98	53.47	85.75
100	54.28	94.05	67.77	94.67

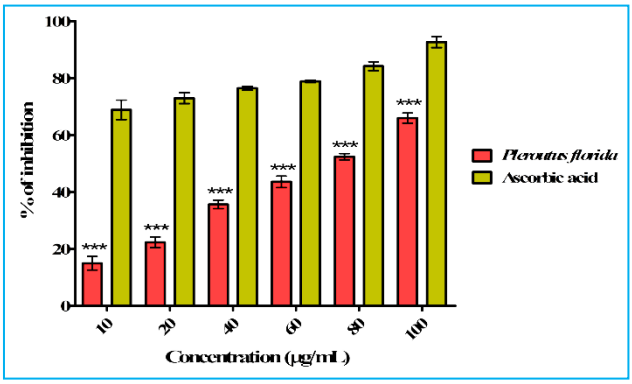

Fig 4 ABTS assay of ethanolic extract of *Pleurotus florida*

In Traditional Medicine, the consumption of shiitake has been suggested for the treatment of several diseases, including cancer [26]. Previous research showed that the addition of horse gramme to the *Pleurotus florida* culture in the paddy straw substrate enhanced the substantial output of mushroom as compared to other investigated supplements [15].

When evaluating a drug's anti-diabetic and antioxidant effectiveness, in vitro tests are crucial screening methods because it may be necessary to screen a large number of prospective treatment candidates [2]. The percentage inhibition arose in a concentration-dependent way in each of the inhibitory experiments. In order to further assess the pharmacological effect of these mushrooms, it would be crucial to take advantage of the concentration-dependent activity of the mushroom extracts [27]. The investigation's findings showed that *Pleurotus florida's* ethanolic extract has antidiabetic and antioxidant properties. Animals, higher plants, and fungus all contain a large number of alpha-amylase inhibitors [1]. To control the activity of these enzymes, these living things produce a broad variety of protein inhibitors of -amylases [16]. The presence of protein and carbohydrates (flavanols), which are thought to be the enzyme's competitive inhibitors, could be the source of the impairment to -glucosidase activity in the extracts of *Pleurotus florida*. This makes sense given that -glucosidase's substrate is a dietary starch and carbohydrate (glycogen) [26].

Also, we looked at *Pleurotus florida* ethanolic extracts for the radical scavenging assays. The effects on the in vitro formation of free radicals were further examined when we discovered that the ethanolic extract of *Pleurotus florida* had the best antioxidant activity [20]. In our investigation, ethanol extracts were used after dried *Pleurotus florida* mushrooms.

The Mushroom *Pleurotus florida* is a good source [21] of extractable phytochemicals that have the ability to block the important enzymes α glucosidase and α amylase, which are connected to Type 2 diabetes mellitus and Antioxidant properties [17]. When screening a large number of possible therapy candidates may be required, in vitro assays can be highly helpful in evaluating a drug's antidiabetic and antioxidant effectiveness [28]. They might offer helpful details regarding the medicinal drugs' mode of action. The findings suggest that the ethanolic extract of *Pleurotus florida* may have antioxidant, antidiabetic, and antiulcer characteristics [18]. This could be because the mushroom contains bioactive compounds.

CONCLUSION

The ethanolic extract of *Pleurotus florida* with its significant antidiabetic and antioxidant activity, suggests its therapeutic potential for the prevention and control of diabetics; moreover, the mushroom species can be used as an easily accessible source of natural antidiabetic and antioxidant and as a possible food supplement or in the pharmaceutical industry.

The suggestion that *Pleurotus florida* could be used as a source of natural antidiabetic and antioxidant compounds, either as a food supplement or in the pharmaceutical industry, aligns with the growing interest in exploring natural products for health benefits. Mushrooms, in particular, have gained attention for their bioactive compounds and potential therapeutic applications. However, more intensive and extensive investigations are needed to exploit their valuable therapeutic potentials and the chemical characteristics of the antidiabetic components in the extracts should be further investigated.

LITERATURE CITED

1. Agrawal D, Chourasia A, Ganeshpurkar A, Shrivastava A, Dubey N. 2022. In vitro α-amylase and α-glucosidase inhibitory potential of *Pleurotus ostreatus* cv. Florida extract. *Indian Journal of Biochemistry and Biophysics* 59: 1016-1019.
2. Ahmed SA, Kadam JA, Mane VP, Patil SS, Baig MMV. 2009. Biological efficiency and nutritional contents of *Pleurotus florida* (Mont.) Singer cultivated on different agro-wastes. *Nature and Science* 7(1): 44-48.
3. Angelini P, Pellegrino RM, Tirillini B, Flores GA, Alabed HB, Ianni F, Ferrante C. 2021. Metabolomic profiling and biological activities of *Pleurotus columbinus* Quél. Cultivated on different agri-food byproducts. *Antibiotics* 10(10): 1245.
4. Bello M, Oluwamukomi MO, Enujiugha VN. 2017. Anti-diabetic activity of three species of oyster mushroom. *Annals: Food Science and Technology* 18(2): 247-254.
5. Brand-Williams W, Cuvelier ME, Berset CLWT. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology* 28(1): 25-30.
6. De Obeso Fernandez Del Valle A, Scheckhuber CQ. 2021. From past to present: Biotechnology in Mexico using algae and fungi. *Plants* 10(11): 2530.
7. Dos Reis EE, Schenkel PC, Camassola M. 2022. Effects of bioactive compounds from *Pleurotus* mushrooms on COVID-19 risk factors associated with the cardiovascular system. *Journal of Integrative Medicine* 20(5): 385-395.
8. Ferraro V, Venturella G, Pecoraro L, Gao W, Gargano ML. 2022. Cultivated mushrooms: Importance of a multipurpose crop, with special focus on Italian fungiculture. *Plant Biosystems* 156(1): 130-142.
9. Flores GA, Girometta CE, Cusumano G, Pellegrino RM, Silviani S, Bistocchi G, Angelini P. 2023. Diversity of *Pleurotus spp*. (Agaricomycetes) and their metabolites of nutraceutical and therapeutic importance. *International Journal of Medicinal Mushrooms* 25(6): 1-20.
10. Hassan SSAMA, Mohamad SA, Ismail R, Rahman SA. 2022. Antioxidant and antimicrobial properties of exo and endo extracts of white oyster mushroom (*Pleurotus florida*). *European Chemical Bulletin* 11(9): 1-5.
11. Khan MA, Tania M. 2012. Nutritional and medicinal importance of *Pleurotus* mushrooms: an overview. *Food Reviews International* 28(3): 313-329.
12. Khatun S, Islam A, Guler P, Cakilcioglu U, Chatterjee NC. 2013. Hypoglycemic activity of a dietary mushroom *Pleurotus florida* on alloxan induced diabetic rats. *Biyolojik Çeşitlilik ve Koruma* 6(2): 91-96.
13. Madaan S, Jabar SIA, Panda BP. 2022. Fatty acids of *Pleurotus florida* mushroom: Potential molecules for blood glucose control. *Food Bioscience* 46: 101558.
14. Menaga D, Rahman PK, Rajakumar S, Ayyasamy PM. 2021. Antioxidant and cytotoxic activities of a novel isomeric molecule (PF5) obtained from methanolic extract of *Pleurotus florida* mushroom. *Jr. of Bioresources and Bioproducts* 6(4): 338-349.
15. Mondal SR, Rehana MJ, Noman MS, Adhikary SK. 2010. Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *Journal of the Bangladesh Agricultural University* 8(2): 213-220.
16. Nantia AE. 2022. In vitro screening of the anti-diabetic activity of six species of edible termite associated mushrooms (*Termitomyces spp*.) from the Western highlands of Cameroon. *Current Research in Environmental and Applied Mycology* (*Journal of Fungal Biology*) 12(1): 125-135.
17. Naraian R, Sahu RK, Kumar S, Garg SK, Singh CS, Kanaujia RS. 2009. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *The Environmentalist* 29: 1-7.
18. Neupane S, Thakur V, Bhatta B, Pathak P, Gautam BB, Aryal L. 2018. Performance of different substrates on the production of oyster mushroom (*Pleurotus florida*) at Gokuleshwor, Darchula. *Int. Jr. Scientific and Res. Publications* 8(6): 231-240.
19. Nkadimeng SM, Nabatanzi A, Steinmann CM, Eloff JN. 2020. Phytochemical, cytotoxicity, antioxidant and anti-inflammatory effects of *Psilocybe natalensis* magic mushroom. *Plants* 9(9): 1127.
20. Pandey K, Ghosh SK, Sanyal T, Bera T, Pal S. 2023. Mycochemistry, antioxidant content, and antioxidant potentiality of the ethanolic extract of *Pleurotus florida* and its anti-cancerous effect on HeLa cancer cell line, and antitumor effect on HeLa-implanted mice. *International Journal of Health Sciences* 17(1): 18-35.
21. Reis FS, Martins A, Vasconcelos MH, Morales P, Ferreira IC. 2017. Functional foods based on extracts or compounds derived from mushrooms. *Trends in Food Science and Technology* 66: 48-62.
22. Shreya S, Jain SK, Guru SK, Sahu AN. 2023. Anti-cancer potential of pleurotus mushroom: Detailed insight on the potential bioactive molecules, in vitro-in vivo studies, and formulation. *Letters in Drug Design and Discovery* 20(4): 439-456.
23. Shreya S, Mohapatra D, Naik GG, Bobde Y, Ghosh B, Sahu AN. 2023. In vitro antioxidant and cytotoxic potential of pleurotus mushroom and activity-based correlation: A comparative study. *Journal of Analytical Chemistry* 78(4): 456-463.
24. Stajic M, Galic M, Cilerdzic J. 2023. Mushrooms–from traditional remedies to the modern therapeutics. *In*: *Mushrooms with Therapeutic Potentials: Recent Advances in Research and Development*. Singapore: Springer Nature Singapore. pp 147-188.
25. Stastny J, Marsik P, Tauchen J, Bozik M, Mascellani A, Havlik J, Kloucek P. 2022. Antioxidant and anti-inflammatory activity of five medicinal mushrooms of the genus Pleurotus. *Antioxidants* 11(8): 1569.
26. Toros GH, El-Ramady H, Prokisch J. 2022. Edible mushroom of *Pleurotus spp*.: a case study of oyster mushroom (*Pleurotus ostreatus* L.). *Environment, Biodiversity and Soil Security* 6(2022): 51-59.
27. Vergun OM, Rakhmetov DB, Shymanska OV, Rakhmetova SO, Fishchenko VV. 2019. Antioxidant activity of seed extracts of selected forage plants. *Plant Introduction* 82: 71-76.
28. Wan LS, Chen CP, Xiao ZQ, Wang YL, Min QX, Yue Y, Chen J. 2013. In vitro and in vivo anti-diabetic activity of *Swertia kouitchensis* extract. *Journal of Ethnopharmacology* 147(3): 622-630.