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DETERMINATION OF *IN-VITRO* ANTIDIABETIC ACTIVITY OF FOXTAIL MILLET (*SETARIA ITALICA*)

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ABSTRACT

Diabetes mellitus is a disorder that occurs when there is little or not enough insulin production from the pancreas. The onset and progression of long-term complications in diabetes mellitus appear to be related to the degree of hyperglycemia and the overall metabolic control. The core remedy for managing diabetes is to lower hyperglycaemia and reduce intestinal glucose absorption through the inhibition of carbohydrate metabolizing enzymes *viz.* alpha-amylase and alpha-glucosidase. Thus, we aimed to determine antidiabetic potential of Foxtail millet by using *in-vitro* experimental methods *viz.* inhibition of carbohydrate hydrolyzing enzymes like alpha-amylase and alpha-glucosidase. Results revealed that the aqueous *(aq.)* Foxtail millet extract at a concentration range of 25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL showed alpha-amylase inhibition effect of 30.87%, 51.79%, 66.98%, and 77.01% respectively with an IC_{50} value of 48.55µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 32.75µg/mL. Similarly, *aq.* Foxtail millet extract at a concentration range of 25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL showed alpha-glucosidase inhibition effect of 25.39%, 40.25%, 56.54%, and 68.97% respectively with an IC_{50} value of 68.75µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 36.45µg/mL. In conclusion, Foxtail millet has potential to subside diabetes mellitus through inhibition of carbohydrate hydrolyzing enzymes such as alpha- amylase and alpha-glucosidase.

Keywords: Foxtail millet, *Setaria italica*, Diabetes mellitus, Alpha-amylase, Alpha-glucosidase

INTRODUCTION

Diabetes mellitus, a chronic, progressive condition associated with multiple metabolic disorders is caused either by the body's inability to produce adequate amounts of insulin or lack of response to the produced insulin or both.[1] This insulin deficiency leads to an increase in the concentration of glucose in the blood and these damages many systems in the body, especially blood vessels and nerves.[2] Hyperglycemia caused by a decrease in insulin production is called type 1 diabetes and hyperglycemia caused by insufficient use of insulin is called type 2 diabetes. Of these two types, type 2 diabetes is a major problem today.[3,4]

Type 2 diabetes is one of the global public health concerns in the 21st century, in both developed and the developing countries are experiencing increasing rates of diabetes.[1] According to the International Diabetes Federation (IDF), a total of 387 million people were diagnosed with diabetes worldwide in 2014, with the figure expected to rise to 592 million by 2035.[5] Diabetes mellitus is a lifelong endocrine disease,[6] and which leads to hyperglycemia and severe irreversible microvascular and macrovascular complications that affect the eyes (diabetic retinopathy), feet (diabetic foot), nerves (diabetic neuropathy), kidneys (diabetic nephropathy), blood vessels (atherosclerosis), and heart (cardiovascular disease). Hence, management of hyperglycemia is of utmost importance to limit the severe complications of diabetes mellitus.[7]

Medicinal plants have been integral to man's health and healing since the dawn of human civilization. Despite of significant advancements made in allopathic medicines during the 20th century, both the ancient and contemporary systems of medicine continue to rely heavily on plants as a source of medications.[8] The majority of the world's populations, who reside in underdeveloped nations, rely on traditional medicine and herbal remedies to meet their primary healthcare needs.[9,10] Furthermore, allopathic drugs used for the treatment of diabetes have their own side effect and adverse effects like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, dyspepsia, dizziness, and joint pain.[8,11] So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect and adverse effects.[8,12]

Ayurveda is an ancient Indian form of medicine which deals with plants and plant extracts. Plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones. Many herbs have been shown to have hypoglycaemic action in animals and humans. *Setaria italica* belongs to family Poaceae commonly known as Foxtail millet. The edible part of the plant is seed. The millet has higher protein and fiber content compared to other millets. In previous studies antihyperglycemic and hypolipidemic activity of aqueous seed extract of *Setaria italica* in streptozotocin induced diabetic rats was reported,[13] with improved activities of carbohydrate metabolic and antioxidant enzymes.[14] With this scenario, in the current study we aimed to determine antidiabetic potential of Foxtail millet by *in-vitro* experimental methods.

MATERIALS AND METHODS

Collection of Foxtail Millet

The commercially available Foxtail millet (Figure 1) was procured from local general store from Bengaluru, Karnataka, India in a clean and sterile plastic container. The collected samples of Foxtail millet were washed thoroughly in running tap water to remove dust and soil particles and were blotted dry. Clean and dried Foxtail millet were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

Figure 1. Showing commercial Foxtail millet

Extract Preparation

Approximately 100g of dried and coarsely powdered Foxtail millet was soaked in a glass jar for 48h at room temperature and solvent was filtered. This process was repeated 3-4 times until filtrate gave no coloration. The filtrate was concentrated to dryness under reduced pressure in rotavapor and finally freeze dried.[15]

Proximate Composition Analysis

The Foxtail millet was analyzed for the proximate composition *viz.* moisture, protein, fat, ash, and crude fiber as per the methods described in Association of Official Analytical Chemists (AOAC, 2005).[16] Nutrients were expressed on dry weight basis.

Moisture content

Moisture content was determined as per the method described in AOAC (2005)[16] as follows:

- Dry the empty dish and lid in the oven at 105°C for 3h and transfer to desiccator to cool. Weigh the empty dish and lid.
- Weigh about 5g of Foxtail millet sample to the dish. Spread the sample to the uniformity
- Place the dish with sample in the oven. Dry for 3h at 105°C.
- After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Moisture content was calculated using following formula:

Moisture content (%) = (Initial weight (g) – Final weight (g)/Weight of sample (g) X 100

Crude fat

The fat content was determined as per procedure described in AOAC (2005).[16] Soxhlet apparatus was used to determine crude fat content of the Foxtail millet samples.

Procedure:

- Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
- Weigh about 3-5g of Foxtail millet sample to paper filter and wrap.
- Take the sample into extraction thimble and transfer into Soxhlet.
- Fill petroleum ether about 250 mL into the bottle and take it on the heating mantle.
- Connect the Soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
- Heat the sample about 14 h (heat rate of 150 drop/min).
- Evaporate the solvent by using the vacuum condenser.
- Incubate the bottle at 80-90°C until solvent is completely evaporated and bottle is

completely dry.

- After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

The percentage of crude fat was calculated using the following formula:

Crude fat (%) = Weight of ether extract (g)/Weight of sample (g) X 100

Protein

Protein content was determined as per procedure described in AOAC (2005)[16] as follows:

- Place the Foxtail millet sample (0.5-1.0g) in digestion flask.
- Add 5g Kjedahl catalyst and 200mL of conc. H_2SO_4.
- Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently until frothing ceases. Boil briskly until solution clears.
- Cool and add 60 mL of distilled water cautiously.
- Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
- Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Percentage of nitrogen and protein was calculated by the following equations:

Nitrogen (%) = T_S-T_B x *Normality of acid* x *0.014)/ Weight of sample (g) X 100*

Where,

T_S - Titre volume of the sample (ml)

T_B - Titre volume of Blank (ml),

0.014-M eq. of N

Protein (%) = Nitrogen x *6.25*

Where,

6.25-The protein-nitrogen conversation factor

Ash content

Drying the Foxtail millet sample (5g) at 100°C and churned over an electric heater. It was then ashes in muffle furnace at 550°C for 5hrs.[16] Ash content was calculated using the following formula:

Ash content (%) = Weight of ash (g)/Weight of sample (g) X 100

Total carbohydrate

The total carbohydrate content of the Foxtail millet varieties was determined as total carbohydrate by difference, calculated by subtracting the measured protein, fat, ash and moisture from 100.[16]

Inhibition Assays of Carbohydrate Hydrolyzing Enzymes

Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay was carried out by the method of Miller, (1959).[17] Different concentrations of *aq.* Foxtail millet extract (25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL) and standard drug acarbose were incubated for 10 minutes at 25°C with 500µL of 20mM sodium phosphate buffer (pH 6.8) with 20µL of amylase (1U/mL). After pre-incubation, each tube was added with 1ml of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) and incubated for 15min. One mL DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10mL) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation of the control samples. The following formula was used to determine the percent inhibition of alpha-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition assay was carried out as described by Matsui et al (1996) with slight modifications.[18] The different concentrations of *aq.* Foxtail millet extract and standard drug acarbose (25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL) were prepared. Phosphate buffer (1 mL; 100mM, pH 6.8) and 80 µL of test *aq.* Foxtail millet extract / acarbose were added to 20 µL of alpha-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG-50µl (5mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5mL of 0.1M Na_2CO_3. The absorbance was measured at 400nm to determine the activity of alpha-glucosidase activity. The following formula was used to determine the percent inhibition of alpha-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

RESULTS

Proximate composition of Foxtail millet

The proximate composition of Foxtail millet was found to be 10.65% moisture, 2.49% crude fat, 74.10% carbohydrates, 12.01% proteins, 0.75% ash, and 2.15% crude fiber (Table 1).

Table 1. Proximate composition of *aq.* Foxtail Millet extract

Proximate Composition	Aq. Foxtail Millet Extract
Moisture, %	10.65
Crude fat, %	2.49
Carbohydrate, %	74.10
Protein, %	12.01
Ash, %	0.75
Crude fiber, %	2.15

Values were expressed as Mean; n=3

Alpha-amylase inhibitory assay

The *aq.* Foxtail millet extract at a concentration range of 25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL showed alpha-amylase inhibition effect of 30.87%, 51.79%, 66.98%, and 77.01% respectively with an IC_{50} value of 48.55µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 32.75µg/mL (Table 2).

Table 2. Effect of *aq.* Foxtail millet extract on alpha-amylase inhibition activity

Conc. of *Aq.* Foxtail millet Extract (µg/mL)	Inhibition (%)	Conc. of Acarbose (µg/mL)	Inhibition (%)
25	30.87	25	48.38

50	51.79	50	69.3
75	66.98	75	84.49
100	77.01	100	94.52
IC_{50} (ug/mL) = 48.55		IC_{50} (ug/mL) = 32.75	

Values were expressed Mean; n=3

Alpha-glucosidase inhibitory assay

The *aq.* Foxtail millet extract at a concentration range of 25µg/mL, 50µg/mL, 75µg/mL and 100µg/mL showed alpha-glucosidase inhibition effect of 25.39%, 40.25%, 56.54%, and 68.97% respectively with an IC_{50} value of 68.75µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 36.45µg/mL (Table 3).

Table 3. Effect of *aq.* Foxtail millet extract on alpha-glucosidase inhibition activity

Conc. of Aq. Foxtail millet Extract (µg/mL)	Inhibition (%)	Conc. of Acarbose (µg/mL)	Inhibition (%)
25	25.39	25	42.90
50	40.25	50	57.76
75	56.54	75	74.05
100	68.97	100	86.48
IC_{50} (ug/mL) = 68.75		IC_{50} (ug/mL) = 36.45	

Values were expressed Mean; n=3

DISCUSSION

Ayurvedic medicine has long employed a number of herbs to treat a variety of ailments.[2] Medicinal plants are the best source of bioresources for chemical entities for synthetic drugs, food supplements, modern and traditional medicine, nutraceuticals, and pharmaceutical intermediates.[19,20] Diabetes is an important human ailment afflicting many from various walks of life in different countries. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[8] Literature reports evidenced that antihyperglycemic and hypolipidemic activity of aqueous seed extract of *Setaria italica* in streptozotocin induced diabetic rats was reported,[13] with improved activities of carbohydrate metabolic and antioxidant enzymes.[14] Therefore, in this study we aimed for determination of antidiabetic activities of *aq.* extract of Foxtail millet by using *in-vitro* experimental methods.

In vitro studies in all fields of biology are aimed at elucidating the mechanisms by which

biological substances perform their roles within a cell.[21] Digestive enzymes such as alpha-glucosidase and alpha-amylase convert starch into glucose and maltose in the intestine. Therefore, the inhibitors of such enzymes are used to manage Type-II diabetes.[1] Concurrently, in our study the *aq.* Foxtail millet extract showed alpha-amylase and alpha-glucosidase inhibition activities with an IC_{50} value of 48.55µg/mL, and 68.75µg/mL respectively. The digestive enzymes such as alpha-glucosidase and alpha-amylase inhibition activities observed in our study was at par with that of standard antidiabetic drug acarbose.

It was understood from the literature that antidiabetic effects of *aq.* extract Foxtail millet could be due to the fact that antihyperglycemic agents present Foxtail millet would have been concentrated in extracts. Moreover, according Krishna Kumari, et al., the hypoglycemic effects of plants may be due to insulin-like substances present in plants,[22] stimulation of beta-cells to produce more insulin,[23] increasing glucose metabolism,[24] or regenerative effect of plants on pancreatic tissue.[25]

CONCLUSION

The findings of our study conclusively demonstrated to possess *in-vitro* alpha-amylase and alpha-glucosidase activities of *aq.* extract of Foxtail millet. Hence, Foxtail millet has potential to subside diabetes through inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase.

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