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Evaluation of *in vitro* antidiabetic activity of two different varieties of finger millet (*Eleusine coracana* - Ragi) and its formulation

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Abstract
Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycaemia leading to acute and chronic complications. α-amylase and α-glucosidase digest the carbohydrates and increase the postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycemia, and reduce the risk of developing diabetes. However, there is limited information available on the presence of α-amylase and α-glucosidase inhibiting compounds. In the current study, the ethanol extracts of *Eleusine coracana* varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 of ratio C014, C015 flake) were tested for α-amylase and α-glucosidase inhibiting activities *in vitro*. The ethanol extracts of *Eleusine coracana* varieties inhibited the activity of α-amylase and α-glucosidase through competitive inhibition and compared with standard as Acarbose. The order of antidiabetic activity was observed as C014:C015 flake, C014:C015 powder, C014 flake, C014 powder and C015 powder variety. The observed effects may be associated with the presence of flavonoids, saponins, and alkaloids. Among the various *Eleusine coracana* varieties and its formulation, C014 flake variety and C014:C015 flake formulation has potential antidiabetic activities were observed.

Keywords: *Eleusine coracana* varieties, antidiabetic, α-amylase and α-glucosidase

Introduction
Diabetes mellitus is one of the most common chronic diseases in the world. The key features of the disease are impaired body glucose metabolism and chronic hyperglycaemia, which can lead to damage to a range of body parts, including eyes, kidneys, nerves and blood vessels (American Diabetes Association, 2014) [1]. One of the most effective approaches for managing diabetes is to decrease postprandial hyperglycaemia (high blood glucose level after a meal) by inhibiting the carbohydrate hydrolysing enzymes, specifically α-glucosidase and α-amylase, in the digestive system (Sales *et al.*, 2012) [2]. Synthetic therapeutic inhibitors, such as acarbose, miglitol and voglibose, are effective against postprandial hyperglycaemia (Standl *et al.*, 2012) [3]. However, these synthetic inhibitors are often associated with undesirable gastrointestinal side effects, such as diarrhea and bloating (Chiasson *et al.*, 2002) [4]. Researchers are seeking alternatives from natural sources with fewer side effects and cost-effective treatment, where more than 1000 plant species have been studied for the treatment of diabetes (Coman *et al.*, 2012) [5]. In particular, millet that are rich in phenolic phytochemicals are gaining increasing attention due to their potential health benefits and their leading role as a staple food in the human diet (Venn and Mann, 2004; Kim *et al.*, 2011) [6, 7]. In the present study, the ethanol extracts of *Eleusine coracana* varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were tested for α-amylase and α-glucosidase inhibiting activities *in vitro*.

Materials and Methods
Collection of finger millet and preparation of powder and flakes
Finger millet varieties as C014 (Bill No. 077950) and C015 (Bill No. 077951) purchased from Tamil Nadu Agricultural University, Tiruvannamalai, Tamil Nadu, India. Before the experiment, dried whole grain of finger millets were ground into powder using a blender. In preparation of flakes, 200gms of powdered finger millet mixed with green chilles (10gms), small onion (20gms), cumin seeds (5gms) and curry leaves (10gms). Required amount of salts were added.Further the mixed sample was grinded, cooked and then kept in overnight. After the overnight, 20ml of buttermilk was added and mixed well. Finally put in the mould and dried the sample under sunlight to make a flake.

Extraction of finger millet powder and flakes
The powdered finger millet were separately weighed by sensitive digital weighing balance and a total of 20g of each variety finger millet (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were macerated with ethanol (20 g in 150 mL) in Erlenmeyer flask for 24 hrs at room temperature (25–27°C). The extraction process goes on for 24 hours facilitated by using shaker. After 24 hrs, the extract was separated from the marc using gauze and further filtered by Whatman filter paper No. 1. The obtained filtrates were concentrated water bath set at 40°C. After drying, the amount of dry extract obtained was

harvested and the dried extract was transferred into airtight bottles and stored in a refrigerator at −4°C until used.

In vitro antidiabetic activity of finger miller varieties and its formulation

In vitro α-amylase and α-glucosidase inhibition assays were carried out by the method of Apostolidis (2007) [8]. Various concentrations of finger millet variety and its formulation extracts were prepared i.e; 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml &, 500 µg/ml using phosphate buffer (pH 6.9). 500 µl of 20% (v/v) each finger millet variety and its formulations and 500 µl of 20 mM phosphate buffer pH 6.9, containing α-amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre-incubation, 1000 µl of 0.5% starch solution in 20 mM phosphate buffer, pH 6.9, was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 500 µl of 96 mM 3, 5-dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temp. Absorbance (A) was measured at 540 nm. In glucosidase, 100 µl of α-glucosidase solution were incubated at 25°Cfor 10 min followed by the addition of 50µl of Paranitrophenyl-α-D-glucopyranoside solution in 0.1 mol l-1phosphatebuffer (pH 6.9). The reacting mixture was then incubated at 25°C for 5 min and the absorbance was read at 405 nm. Acarbose was used as positive control and the inhibitory activity of α- amylase and α-glucosidase and percent of inhibition was calculated.

Statistical analysis

All experiments were performed in triplicates. Means and standard deviations were calculated from replicates within the experiments. The half inhibition concentration (IC50) was calculated using graph pad prism software.

Results and discussion

α -amylase inhibition assay

Inhibition of α-amylase activity by finger millet variety and its formulation extracts and Acarbose was found to be dose dependent from 100 to 500 µg/ml concentrations (Table 1 and Fig. 1). A maximum of 77.08, 76.34, 80.78, 79.51% inhibition of α-amylase activity was observed at 500µg/ml concentration for the powder of C014, C015, flake of C014, C015 while 82.78, 86.58 and 91.44% in formulation of 1:1 ratio of C014: C015 powder, 1:1 ratio of C014: C015 flake and standard Acarbose respectively. The IC_{50} was 326.57, 347.93, 303.93 and 311.67 µg/ml for the powder of C014, C015, flake of C014, C015) while 291.36, 279.78 and 258.77 µg/ml in formulation 1:1 ratio of C014, C015 powder, 1:1 ratio of C014, C015 flake and standard Acarbose, respectively. The lowest IC_{50} values has highest antidiabetic activity.

Table 1: *In vitro* antidiabetic activity of the ethanol extract of two different finger millet varieties and its formulation using alpha amylase enzyme and comparison with standard drug Acarbose

% of inhibitions	Concentrations (µg/ml)					IC_{50} (µg/ml)
	100	200	300	400	500	
Ragi powder CO14	14.57±1.01	24.70±1.72	47.62±3.33	64.20±4.49	77.08±5.39	326.57
Ragi powder CO15	10.77±0.75	19.42±1.35	45.30±3.17	57.55±4.02	76.34±5.34	347.93
Ragi powder formulation (CO14: CO15)	18.79±1.31	34.84±2.43	52.27±3.65	68.21±4.77	82.78±5.79	291.36
Ragi flakes CO14	17.74±1.24	31.25±2.18	50.26±3.51	66.73±4.67	80.78±5.65	303.93
Ragi flakes CO15	16.05±1.12	28.40±1.98	50.05±3.50	66.42±4.64	79.51±5.56	311.67
Ragi flakes formulation (CO14: CO15)	20.06±1.40	36.53±2.55	53.85±3.76	69.79±4.88	86.58±6.06	279.78
Std. (Acarbose)	23.86±1.67	39.28±2.74	57.55±4.02	72.65±5.08	91.44±6.40	258.77

Values expressed as Mean ± SD for triplicates

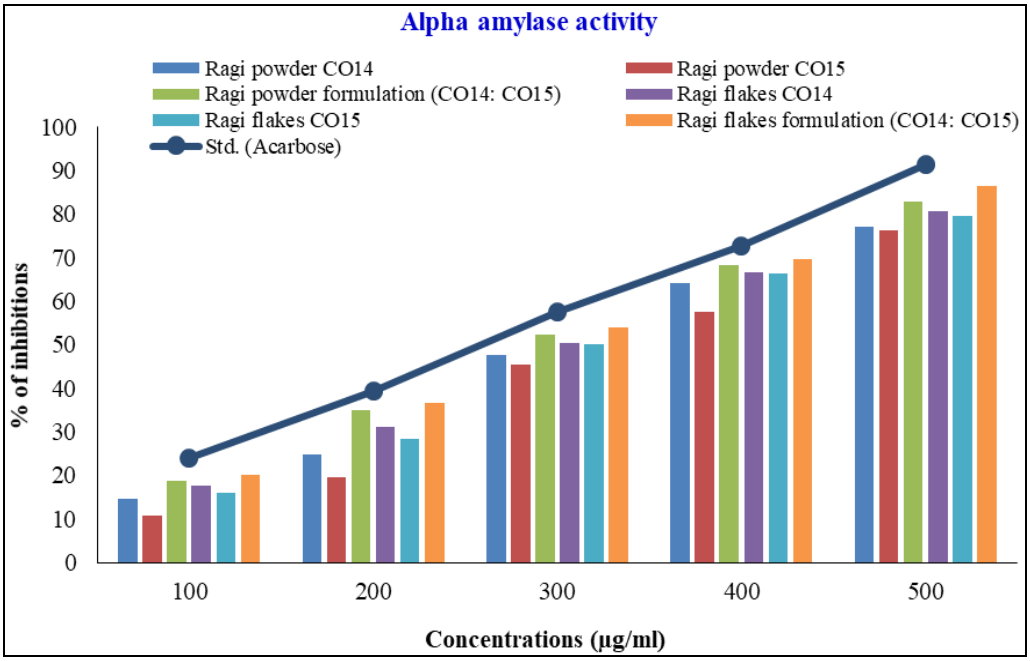

Fig 1: *In vitro* antidiabetic activity of the ethanol extract of two different finger millet varieties and its formulation using alpha amylase enzyme and comparison with standard drug Acarbose

α-glucosidase inhibition assay

Inhibition of α-glucosidase activity by finger millet variety and its formulation extracts and Acarbose was found to be dose dependent from 100 to 500 µg/ml concentrations (Table 2 and Fig. 2). A maximum of 75.27, 72.69, 76.93, 76.19% inhibition of α-amylase activity was observed at 500µg/ml concentration for the powder of C014, C015, flake of C014, C015 while 81.36, 83.21 and 92.06% in formulation of 1:1 ratio of C014: C015 powder, 1:1 ratio of C014: C015 flake and standard Acarbose respectively. The IC_{50} was 341.19, 354.55, 320.09 and 332.06µg/ml for the powder of C014, C015, flake of C014, C015) while 298.63, 265.93 and 238.85µg/ml in formulation 1:1 ratio of C014, C015 powder, 1:1 ratio of C014, C015 flake and standard Acarbose, respectively.

The lowest IC_{50} value has highest antidiabetic activity. The order of antidiabetic activity was observed as C014:C015 flake, C014:C015 powder, C014 flake, C014 powder and C015 powder variety. Among the various *Eleusine coracana* varieties and its formulation, C014 flake variety and C014:C015 flake formulation has potential antidiabetic activities were observed.

Table 2: *In vitro* antidiabetic activity of the ethanol extract of two different finger millet varieties and its formulation using alpha glucosidase enzyme and comparison with standard drug Acarbose

% of inhibitions	Concentrations (µg/ml)					IC_{50} (µg/ml)
	100	200	300	400	500	
Ragi powder CO14	13.65±0.95	27.67±1.93	37.82±2.64	62.91±4.40	75.27±5.26	341.19
Ragi powder CO15	13.09±0.91	24.90±1.74	36.34±2.54	60.70±4.24	72.69±5.08	354.55
Ragi powder formulation (CO14: CO15)	20.47±1.43	35.05±2.45	48.70±3.40	65.49±4.58	81.36±5.69	298.63
Ragi flakes CO14	17.52±1.22	31.36±2.19	44.46±3.11	64.39±4.50	76.93±5.38	320.09
Ragi flakes CO15	15.31±1.07	28.78±2.01	41.32±2.89	63.28±4.42	76.19±5.33	332.06
Ragi flakes formulation (CO14: CO15)	21.21±1.48	41.14±2.87	59.22±4.14	71.58±5.01	83.21±5.82	265.93
Std. (Acarbose)	24.90±1.74	44.83±3.13	62.36±4.36	76.56±5.35	92.06±6.44	238.85

Values expressed as Mean ± SD for triplicates

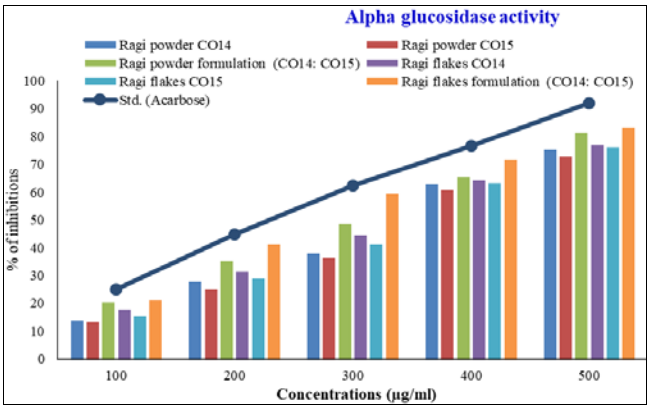

Fig 2: *In vitro* antidiabetic activity of the ethanol extract of two different finger millet varieties and its formulation using alpha glucosidase enzyme and comparison with standard drug Acarbose

Diabetes is the major health problem and continues to be one of the major causes of the death all over the world. Various therapeutic agents are available in medicine to treat diabetes, but they are toxic, expensive and associated with many side effects (Apostolidis *et al.*, 2007) [8]. The alphaamylase enzyme is known as one of the key enzymes in a human digestive system which converts starch to monosaccharide and causes the rise in the blood glucose (Sunila *et al.*, 2012) [9]. Amylase acts upon large polysaccharides (starch) at internal bands. The inhibition of alphaamylase has been suggested as a strategy for diabetes and obesity management by reducing sugars levels in the blood. Although modern medicines have introduced many synthetic therapeutic agents like insulin, biguanides, sulfonylureas and thiazolidinedione are to treat diabetes but still there are no any satisfactory drugs to avoid diabetic complications (Oboh *et al*., 2012) [10].

Traditional medicinal plants having anti-diabetic properties can provide useful sources for the discovery of safer hypoglycemic agents (Yao *et al*., 2013) [11]. These plants are the major source for discovering new compounds with therapeutic value for drug development against most common and very prevalent disease, diabetes mellitus. Enzyme inhibition assay for plant extracts determines the inhibitory potency of the sample against the enzyme, and it is one of the mechanisms through which plant could show its antidiabetic activity. In the present study, the concentrated and dried extracts of finger millet variety (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were evaluated for antidiabetic activity by employing standard *in vitro* techniques (Alpha-amylase and Alpha-glucosidase model). α -amylase and α-glucosidase digest the carbohydrates and increase the postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycemia, and reduce the risk of developing diabetes. Acarbose is the enzyme inhibitors that are currently used for controlling PPHG (Post parental hyperglycemia). Acarbose inhibits both α-amylase and α-glucosidase,

Qualitative phytochemical analysis of finger millet variety and its formulation obtained from multiple extraction solvents detected several phytomolecules including flavonoids, saponins, tannins, alkaloids, and phytosterols. The presence of flavonoids, especially in chloroform, ethanol, methanol, and distilled water extracts, may account for the inhibitory activity observed. Flavonoids, heterogeneous group of plant polyphenols, have widely reported inhibitory activity against *α*-amylase and *α*-glucosidase in both *in vitro* and *in vivo* and *in silico* modelling studies (Spínola *et al.*, 2017; Kazeem *et al.*, 2013) [12, 13]. Importantly, some investigators have reported that there is a positive relationship between total flavonoid and polyphenol content and the ability to inhibit *α*-amylase and *α*-glucosidase (Ramkumar *et al*., 2010) [14]. These results demonstrated the glucose lowering effect of the finger millet variety and its formulation extracts in vivo, possibly due to the α-amylase and α-glucosidase inhibiting activities observed *in vitro*.

Conclusion

In the present study in both *in vitro* methods showed antidiabetic activity of finger millet variety (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were dose dependent manner and overall extracts with a good percentage of inhibition. Among the various finger millet varieties and its formulation, C014 flake variety and formulation of C014:C015 flake has potential antidiabetic activities were observed. The result of this study are encouraging which may offer a safe method or supplement treatment strategy to control diabetes through its alpha-amylase and α-glucosidase inhibition, Therefore; their derived products may be an important source of nutrition and therapy. Further, *in vivo* studies needs to be carried out that can serve in the development of new pharmaceuticals to treat diabetes mellitus.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care,2014:37:81-90.
2. Sales PM, Souza PM, Simeoni LA, Magalhães PO, Silveira D. α-Amylase inhibitors A review of raw material and isolated compounds from plant source. J. Pharm. Pharm. Sci,2012:15:141-183.
3. Standl E, Schnell O. Alpha-glucosidase inhibitors 2012–cardiovascular considerations and trial evaluation. Diab. Vasc. Dis. Res,2012:9:163-169.
4. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Group SNTR, Acarbose for prevention of type 2 diabetes mellitus: The STOP-NIDDM randomised trial. Lancet,2002:359:2072-2077.
5. Coman C, Rugina OD, Socaciu C. Plants and natural compounds with antidiabetic action. Not. Bot. Horticult. Agrobot,2012:40:314-325.
6. Venn BJ, Mann JI. Cereal grains, legumes and diabetes. Eur. J. Clin. Nutr,2004:58:1443-1461.
7. Kim JS, Hyun TK, Kim MJ. The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. Food Chem,2011:124:1647-1651.
8. Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science & Emerging Technologies,2007:8(1):46-54.
9. Sunila C, Agastian P, Kumarappan C, Ignacimuthu S. *In vitro* antioxidant, antidiabetic and antilipidemic activities of *Symplocos cochinchinensis* (Lour.) S. Moore bark. Food Chem Toxicol,2012:50:1547-53.
10. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle TH, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (alpha amylase and alpha glucosidase) and hypertension (angiotensin I converting) *in vitro*. J Funct Foods,2012:4:450-8.
11. Yao X, Ling Zhu L, Chen Y, Tian J, Wanga Y. *In vivo* and *in-vitro* antioxidant activity and a-glucosidase, a-amylase inhibitory effects of flavonoids from *Cichorium glandulosum* seeds. Food Chem,2013:139:59-66.
12. Spínola V, Castilho PC. Evaluation of Asteraceae herbal extracts in the management of diabetes and obesity. Contribution of caffeoylquinic acids on the inhibition of digestive enzymes activity and formation of advanced glycation end-products (*in vitro*) Phytochemistry,2017:143:29-35.
13. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of morinda lucida benth leaf. BioMed Research International, 2013, 6.
14. Ramkumar KM, Thayumanavan B, Palvannan T, Rajaguru P. Inhibitory effect of *Gymnema montanum* leaves on α-glucosidase activity and α-amylase activity and their relationship with polyphenolic content. Medicinal Chemistry Research,2010:19(8):948-961.