Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(3): 105-110

Received: 04-01-2021
Accepted: 08-03-2021

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In vitro α amylase and amyloglucosidase inhibitory activities of selected underutilized cereals, yams and root crops

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DOI: https://doi.org/10.22271/plants.2021.v9.i3b.1271

Abstract
Diabetes is one of the most challenging global health problems. Currently, reducing postprandial blood glucose concentration by inhibiting carbohydrate hydrolyzing enzymes is recognized as an effective treatment against diabetes. In this study, *in vitro* α-amylase and amyloglucosidase inhibitory effect of selected, underutilized and locally grown cereals (*P. miliaceum*, *P. scrobiculatum* and *S. italica*), yams (*D. alata* and *D. esculenta*) and root crops (*L. spinosa* and *N. nucifera*) were evaluated related to their dietary fiber contents. Results showed that the inhibitory effect of selected food crops has a positive correlation with their dietary fiber contents. *L. spinosa* with higher dietary fiber content generally had higher α-amylase (78.0%) and amyloglucosidase (71.28%) enzyme inhibitory percentages among yams and root crops. Significantly highest ($p<0.05$) dietary fiber content was in *S. italic* among three cereal varieties and it showed the highest inhibition activity against α-amylase (67.8%) and amyloglucosidase (59.40%) activity. These tested food crops are considerable sources of dietary fiber and are having α-amylase and amyloglucosidase enzyme inhibitory actions against starch hydrolysis.

Keywords: α amylase inhibition, amyloglucosidase inhibition, cereals, root, yams

1. Introduction
Diabetes mellitus is one of the most common chronic non-communicable diseases characterized by elevated fasting blood glucose levels (≥125 mg/dl) [1]. Elevation of blood glucose concentration occurs either due to pancreatic β cells does not secrete enough insulin enzyme (type I diabetes mellitus) or cells in the human body do not properly respond to the produced insulin (type II diabetes mellitus). American Diabetes Association reported as around 90-95% of identified diabetes cases are related to type II diabetes mellitus (American Diabetes Association [2]). Diabetes mellitus is prevailing all over the world and it is predicted to be one of the main death causes disease in the next two to three decades. The most diagnosed cases are from Asia and Africa region and the accelerating rate of diabetes mellitus is expected to increase by two to three folds by 2030 [3]. It impresses the important and urgent requirement of controlling or preventing diabetes mellitus conditions among people. The control of postprandial blood glucose elevation is one of the efficient methods in the management of diabetes mellitus [4]. The management of post-prandial blood glucose concentration is associated with the inhibition of starch hydrolysis enzymes such as α amylase and amyloglucosidase [5]. α Amylase enzyme hydrolyzes the α 1-4 linkages in starch and convert high molecular weight starch into simple and more absorbable compounds such as glucose, maltose and maltotriose. Subsequently, the amyloglucosidase enzyme is responsible to convert all the simple molecules of starch into glucose [6]. By inhibition of these enzyme activities, it will induce the delaying or decreasing the starch digestion which is important in controlling diabetes mellitus. Some synthetic inhibitors such as acarbose and voglibose are clinically used to reduce the starch hydrolysis enzyme activities to reduce the postprandial blood glucose concentration. However, those synthetic medicines have been shown side effects such as diarrhea, abdominal pain and hypoglycemia and with prolonged treatment, the drug resistance to these medicines has been reported from diabetes patients [3]. Therefore, apart from currently available medications, the naturalmedicines from plant sources have obtained more importance, since they are not much expensive and free from side effects compared to synthetic diabetes control drugs [5]. In Sri Lanka, there are plenty of underutilized food crops importantly cereals, yams, and tuber crops which are not much systematically exploited regarding the anti-diabetes activity.

Cereals, yams, and root crops contained several types of phytochemicals which are reported to having therapeutic effects against diabetes mellitus [7-10]. Whole grain cereals, yams and root crops have consisted of dietary fiber, including soluble dietary fiber and insoluble dietary fiber [11]. Several researchers have documented the relationship between dietary fiber intake and a decrease in glycemic response in diabetes mellitus [12]. This study will reflect the effect of the dietary fiber content of selected food crops on *in vitro* α amylase and amyloglucosidase enzyme inhibition action with the means of controlling diabetes mellitus. The findings may be important when applying those selected food crops in functional food formulations for delivering additional health benefits to the consumers.

2. Materials and Methodology

2.1 Materials

Three yam varieties of *D. alata* (Kahata-ala/KA, & Hingurala/H) and *D. esculenta (*Java-ala/JA) were harvested from Hambantota area in Sri Lanka and *N. nucifera* (Lotus root) and *L. spinosa* (*Lasia* root), *Panicum miliaceum* (Proso millet), *Paspalum scrobiculatum* (Kodo millet) and *Setaria italica* (Foxtail millet) were purchased from the local market in Hambantota area in Sri Lanka. Porcine pancreatic α amylase, amyloglucosidase, dinitro salicylic acid, sodium hydroxide, sodium potassium tartrate, sodium phosphate, disodium orthophosphate, acetone, ethanol, sodium chloride and celite were purchased from Sigma (Sigma Co., St. Louis, MO, USA). All the chemicals and reagents procured were analytical grade.

2.2 Sample preparation

All three cereal grains (*P. miliaceum, P. scrobiculatum* and *S. italica*) were de-hulled and grounded using a grinder (Philips HL772, Thailand) followed by sifted through 450 µm sieves. All five types of roots and tubers (*D. alata* (KA, & H), *D. esculenta*-JA, *L. spinosa* and *N. nucifera*) were washed, peeled and removed defective parts followed by cut into thin slices (thickness ~ 3 mm) and immediately soaked in 2% citric acid solution for 20 minutes (Bindu, Mythili, & Radhika, 2018) and dried using hot air oven (MEMMERT NLE 500, Germany) at 45 ºC for 48-72 hrs. The dried pieces were grounded using a laboratory-scale grinder (Philips HL772, Thailand) and sifted through a 450 µm sieve. Flour samples were packed and stored at -20 ºC for further analysis.

2.3 Total Dietary fiber analysis

Total dietary fiber content was analyzed using the modified enzymatic-gravimetric procedure as mentioned in Prosky, (1979) [13]. One gram of sample from each was weighed into a 600 mL beaker and 50 mL of phosphate buffer (pH 6.0) was added to each beaker. Then the samples were heated after adding100 µL of heat-stable alpha-amylase at 95 ºC for 15 minutes and then samples were digested with 5.0 mg of protease for 30 minutes at 60 ºC. Then 0.3 mL of amyloglucosidase was added and incubated at 60 ºC for 30 minutes in a water bath. 95% ethanol (four volumes) was added to precipitate dietary fiber and kept overnight. Then the solution part was filtered out through the crucible and precipitate was washed off using 20 mL portions of 78% ethanol, 95% ethanol and acetone two times accordingly. The precipitate was then oven-dried at 105 ºC overnight in a hot air oven (MEMMERT NLE 500, Germany) and then weighed. Values obtained by the enzymatic method were then corrected by analyzing protein and ash in the samples.

2.4 *In vitro* α amylase inhibitory assay

In vitro α amylase activity was measures using the method described in Xiong *et al*., (2020) [14] with some modifications as mentioned in Mel et al (2020) [15] and Janarny and Gunathilake, (2020) [16]. A sample of 1.00 mg from each yam, roots and cereal flours were weighed and 100 µl of the α-amylase enzyme was added which was prepared by adding 27.5 mg of enzyme in 100 mL of 20 mmol sodium phosphate buffer containing 6.7 mmol of sodium chloride was added (pH 6.9). Then the reaction mixture was pre-incubated at 37 °C for 20 min. Then the 1% starch solution was prepared and 100 µl of the starch solution was added into the reaction mixture before incubating it again at 37 °C for 10 min. At the end of the incubation 200 µl of dinitro salicylic acid solution was added to colour development followed by keeping the test tubes in a boiling water bath for 5 min to terminate the reaction. Then the reaction mixture was diluted with 2.20 mL of distilled water and absorbance was read at 540 nm by UV/Visible spectrophotometer (Thermo Scientific 201, United State). The experiments were conducted in triplicates and the absorbance of blank, control was measured.

2.5 *In vitro* amyloglucosidase inhibition assay

The *In-vitro* amyloglucosidase inhibition activity of selected cereals, tubers and root flours was determined according to the method in Si *et al*, (2020) [17] with slight modifications. A sample of 1.00 mg from each yam, roots and cereal flours were weighed and added into test tubes. Freshly diluted amyloglucosidase to 6.5 U/ mL in 0.1M sodium phosphate buffer was added to each tube and followed by incubated in a water bath at 37 °C for 20 min. Subsequently, 20 µL of 1% starch solution was added as the substrate to each reaction mixture and incubated at 37 °C for 10 min. Then 100 µL of dinitro salicylic acid (color reagent) was added into the test tube and incubated at 100 °C for 5 min. Then reaction mixtures were cooled in an ice-water bath before adding 2.5 mL of distilled water to dilute the reaction mixture. The absorbance of each reaction mixture was measured at 540 nm using a UV/Visible spectrophotometer (Thermo Scientific 201, United State). A blank was prepared to replace amyloglucosidase and 1% starch solution with sodium phosphate buffer. Control was prepared by replacing the amyloglucosidase enzyme with sodium phosphate buffer.

2.6 Statistical Analysis

All the analyses were conducted in triplicate and the data were expressed as mean ± standard deviation. The sample means were compared at the 95% confidence level ($p < 0.05$) using the Tukey's test in SPSS 16.0 software.

3. Results and Discussion

The data in Table 1 reflect the dietary fiber contents of *P. miliaceum, P. scrobiculatum, S. italica, D. alata* (KA, & H), *D. esculenta*-JA, *L. spinosa* and *N. nucifera.*

Table 1: Dietary fiber contents of selected cereal, yams and root crops

Crop	Dietary fiber content (g/100 g in dry weight basis)
D. alata-KA	11.75 ± 0.26^{d}
D. alata– H	6.83 ± 0.66^{a}
D. esculenta-JA	36.06 ± 0.37^{f}
L. spinosa	14.36 ± 0.27^{e}
N. nucifera	39.42 ± 0.74^{g}
P. miliaceum	8.70 ± 0.12^{c}
P. scrobiculatum	8.11 ± 0.14^{b}
S. italica	14.18 ± 0.32^{e}

Values expressed as means ± SD (n=3). Different letter superscripts express significant differences between values of the same column ($p \leq 0.05$).

When considering total dietary fiber contents in yams and root crops, *L. spinosa* root (39.42%) and *D. esculenta*-JA (36.06%) showed significantly higher value ($p \leq 0.05$) compared to the other three varieties. It is reported as a mucilaginous fraction of yams that contains soluble glycoprotein and dietary fiber [7]. It was visually observed in *D. esculenta*-JA, containing a higher amount of mucilage and it may be the reason for comparatively higher dietary fiber content. There is evidence showing *L. spinosa* root contain a higher amount of dietary fiber which was compatible with the present study and it has been evaluated the dietary fiber content of *L. spinosa* root using six samples from six different geographical areas and dietary fiber content was in the range of 40-74% in dry weight basis [18]. Another researcher presented the dietary fiber content of *L. spinosa* root as 45.34% that is also linear with our results [19]. The total dietary fiber contents of three *Dioscorea* varieties were in the range of 6.83-36.06%. The previously presented data for total dietary fiber contents of 16 *D. alata* species were in the range of 4.1 to 11% as in Wireko-manu *et al*., (2009) [20] and however, the value obtained for the *D. esculenta*-JA in the present study was much higher (36.06%). The dietary fiber content of *N. nucifera* roots in this study was higher than the value (11.85%) obtained by Ham et al [21]. The total dietary fiber contents of three whole grain millet species were 8.70, 8.11 and 14.18% in *P. miliaceum, P. scrobiculatum* and *S. italica* respectively. When comparing dietary fiber contents of three selected cereals, a significantly higher value was observed in *S. italica.*

The following two graphs (Figure 1 and Figure 2) show the α amylase and amyloglucosidase enzyme inhibition activity% observed from selected three cereal crops and five yams and root crops respectively.

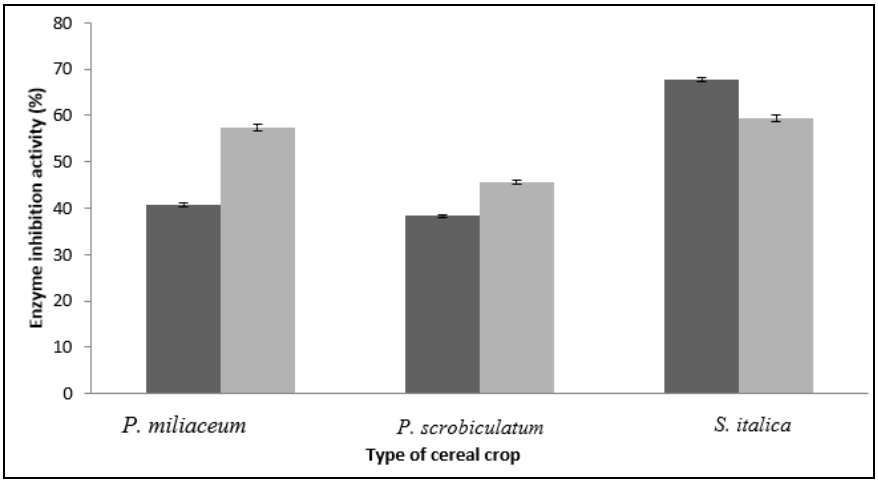

Fig 1: α amylase and amyloglucosidase inhibitory activity of selected cereals; *P. miliaceum*, *P. scrobiculatum* and *S. italica*. (■ - α amylase inhibition%, ■ -Amyloglucosidase inhibition%). The data are indicated as the mean± standard deviation [n=3].

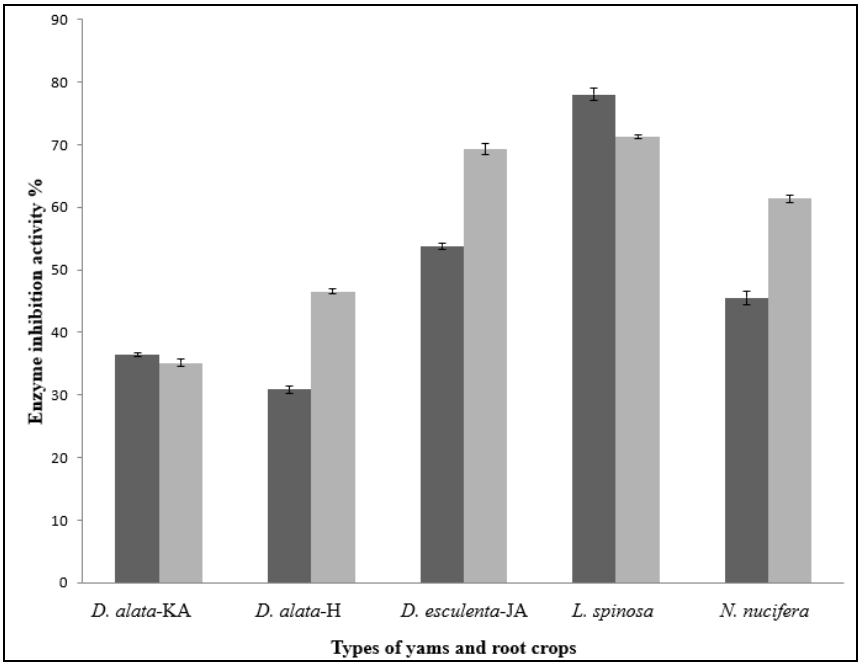

Fig 2: α amylase and amyloglucosidase inhibitory activity of selected yams and root crops (■- α amylase inhibition%, ■-Amyloglucosidase inhibition%).The data are indicated as themean± standard deviation [n=3].

Enzymes are considered as a protein-based biological catalyst which can regulate the specific biochemical reactions. α amylase enzyme found in the human digestive tract can breakdown α, 1-4 glycosidic bonds in the amylose and amylopectin chains in starch. α amylase enzyme can convert above the substrate to oligosaccharides mainly. Since this enzyme act on both amylose and amylopectin, it can produce particular end products as maltotriose and maltose from

amylose and maltose, glucose and limit dextrin from amylopectin [22]. Amyloglucosidase is also a starch hydrolyzing enzyme that cleavage the α 1-4 glycosidic bonds from the non reducing ends of starch and release glucose molecules. Further, amyloglucosidase can catalyze the releasing of β-d-glucoseby cleavage α-1,4 and α-1,6 linkagesin non-reducing ends of starch and maltooligosaccharides to produce glucose [23]. After food intake, the α amylase action will take place and it can increase the postprandial blood glucose level in diabetic patients since α amylase can convert starch into simple and more absorbable forms. This phenomenon can be controlled or regulated by inhibiting the α amylase activity. Apart from using synthetic medicines to achieve that it is very important to reveal the possibility of foods themselves to control diabetes conditions. According to the obtained results for α amylase inhibition activity of selected three cereal varieties, the values were as 40.80±0.43, 38.31±0.21and 67.80±0.48% in *P. miliaceum, P. scrobiculatum* and *S. italica* respectively. According to the obtained results, *S. italica* showed the highest significant percentage ($p \leq 0.05$) of α amylase inhibition activity. *P. miliaceum, P. scrobiculatum* were also showed considerable inhibitory activity. However, only *S. italica* has shown more than 50% inhibition action. The amyloglucosidase inhibition activity of *P. miliaceum, P. scrobiculatum* and *S. italica* was ranged from 45.54±0.45 to 59.40±0.81%. The highest amyloglucosidase inhibition activity was observed in *S. italica* as similar observation in the α amylase inhibition activity while, *P. miliaceum, P. scrobiculatum* have shown 57.42±0.62 and 45.54±0.45% of amyloglucosidase inhibitory action respectively. When considering the *in vitro* enzyme inhibition activities resulted from yams and root crops together, the values were significantly different ($p \leq 0.05$) in both assays. According to the results indicated in Figure 2, all yams and root crop varieties *D. alata-KA, D. alata-H, D. esculenta-JA, L. spinosa* and *N. nucifera* showed considerable inhibition activities. According to the results obtained for α amylase inhibition activity, it was ranged from 30.87±0.54 to 78.00±0.96% and *L. spinosa* showed the highest significant ($p \leq 0.05$) α amylase inhibition activity among tested five yams and root crops samples. Amyloglucosidase enzyme inhibition activities of *D.alata-KA, D.alata-H, D. esculenta-JA, L. spinosa* and *N. nucifera* were as 35.14±0.56, 46.53±0.37, 69.30±0.87, 71.28±0.32 and 61.38±0.54. Percentage inhibition of amyloglucosidase enzyme activity was also significantly higher in *L. spinosa* compared to other yams and roots. The significantly lowest ($p \leq 0.05$) α amylase inhibition activity was found in *D. alata*-H while *D.alata*-KA showed the lowest amyloglucosidase inhibition action. According to a previous study, The ethanolic extracts of *P. miliaceum* and *S. italica*have shown α amylase inhibitory action and they reflect the effect of the phenolic compound on that inhibition potential [24]. Reddy and others investigated α amylase inhibition activity of *P. scrobiculatum* and reported it has remarkable inhibition activity [25]. However, supporting information for the present study's data could not found due to a lack of literature that discussing the α amylase inhibition and amyloglucosidase inhibition activities in terms of dietary fiber content in selected food crops.

Figure 3 and Figure 4 reflect the correlation between dietary fiber content and assessed enzyme inhibitory activities of yams and root crops, and cereal varieties accordingly.

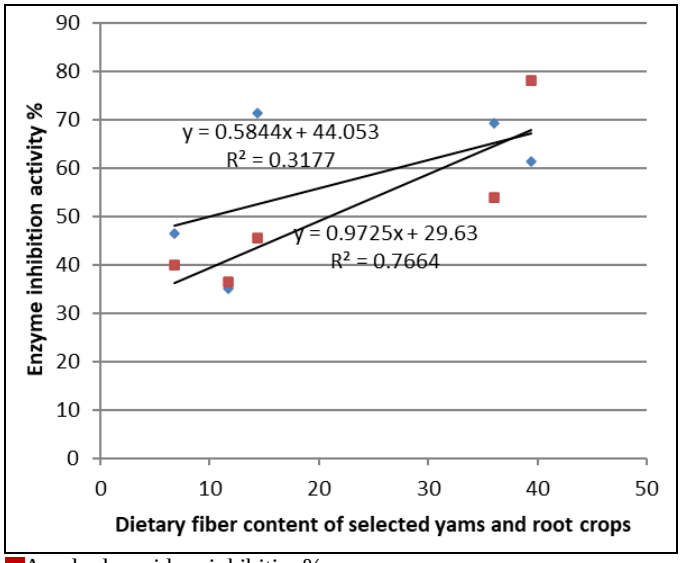

Fig 3: Correlation between dietary fiber content and enzyme inhibition activity% of selected yams and root crops

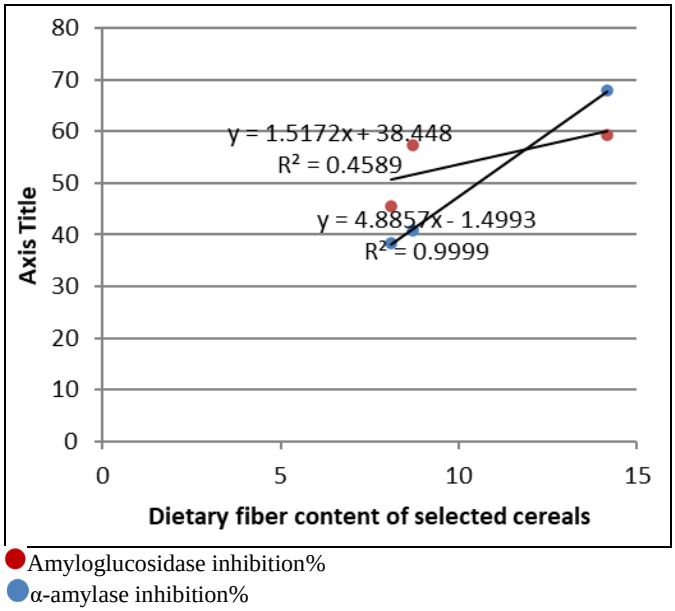

Fig 4: Correlation between dietary fiber content and enzyme inhibition activity% of selected cereal crops

The correlation between dietary fiber content and α amylase and amyloglucosidase enzyme inhibitory percentages were analyzed separately for yams and roots, and cereals. According to the obtained results, the correlation between the dietary fiber content of yams and roots with α amylase inhibitory action showed (R^2 = 0.3177) a positive weak correlation. Meanwhile, the relationship between the dietary fiber content of yams and roots with amyloglucosidase inhibitory action showed a strong positive correlation (R^2 = 0.7664). When considering the correlation pattern of α amylase inhibition activity of *P. miliaceum, P. scrobiculatum* and *S. italica* and their dietary fiber contents, it showed a very strong positive correlation (R^2 = 0.9999) and the correlation regression value for dietary fiber content and amyloglucosidase inhibition activity of tested cereals was R^2 = 0.4589, categorized as a moderate positive correlation.

All four tested correlation patterns were showed positive correlation and their correlation levels were interpreted as weak, strong, very strong and moderate according to Schober and Boer, (2018) [26]. The resulted in positive correlation from tested eight food crops under cereals, yams and roots reflect, the increased dietary fiber content potent to increase the α amylase and amyloglucosidase enzyme inhibition activity. Qi and the group have shown a positive correlation between rice bran insoluble dietary fiber and α amylase inhibitory activity [27]. The influence of wheat bran dietary fiber against α amylase enzyme activity has been reported by Ou *et al*., 2001.There are a few suggested pathways or mechanisms of enzyme inhibition activity by dietary fiber. Meng et al., 2019 reported the positive correlation between dietary fiber of buckwheat straw with α amylase inhibition activity. Ou *et al.*, (2001) [28] reported as dietary fibers can be adsorbed to starch in the food and disturbing the hydrolysis of starch by hydrolytic enzymes such as α amylase and dietary fiber act as a physical barrier in between starch and hydrolysis enzymes which hinder the reaction between starch and enzymes. Several reports discussed the overall capability of reducing the risk of diabetes mellitus by dietary fiber intake and the suggested mechanisms. One of them is by reducing the gut transit time especially by soluble dietary fiber leads to reduce starch digestion in the small intestine and they are transferred into the large intestine quickly [29]. The other view was the dietary fiber can increase the viscosity of content in the small intestine and reduce the accessibility of enzymes to the substrate (starch) [30].

5. Conclusion

In conclusion, our investigation demonstrated that we tested *P. miliaceum, P. scrobiculatum, S. italica, D. alata* (KA, & H), *D. esculenta*-JA, *L. spinosa* and *N. nucifera* exhibited significant *in vitro* inhibitory activity against α-amylase and amyloglucosidase enzymes. The extent of inhibition was related to the dietary fiber contents. *L. spinosa* sample with higher dietary fiber content generally had significantly higher ($p \leq 0.05$) α-amylase and amyloglucosidase enzyme inhibitory percentages among yams and root crops. Significantly highest ($p < 0.05$) inhibition activities against α-amylase and amyloglucosidase were shown by *S. italica* sample and the highest dietary fiber content also was in *S. italica* among three cereals. The findings may important to generalize these selected underutilized sources as good dietary fiber sources and their potential enzyme inhibitory activity against starch hydrolysis which leads to reduce the risk of diabetes mellitus. Further, incorporation of these dietary fiber sources into food formulation and producing functional food products may lead to giving additional health benefits to the consumers. However, further investigation needs to be done related to the hypoglycemic activity of these crops by different *in vitro* and *in vivo* assays to confirm their anti-diabetic potential and action.

6. Acknowledgements

We greatly acknowledge the financial support provided by World Bank AHEAD project under the research grant AHEAD/RA3/DOR/WUSL/FST.

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