Nutritional analysis and glucosidase inhibition activity of kodo millet

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INTRODUCTION

Kodo millet (Paspalum scrobiculatum) is widely distributed across the tropics and subtropics of the world. It is native cereal of India and consumed as healthy food in all other parts of India especially in southern part of India [1]. It has annual production of about 310,710 tonnes [2]. Kodo millet are closely resembles the rice and helps to use in weight loss. It is rich in phytochemicals which helps in preventing different lifestyle related diseases and also helps in reducing the joint pain [3]. Kodo millet flour can be used for novel foods like idli, dosa, biscuits and bread [4]. Diabetes is the world’s fastest-growing metabolic disorder in recent years that are affecting the world population of various age group. This is due to the poor nutrition availability and living a sedentary life style. To control the diabetes in early stage involves the inhibition of the carbohydrate-hydrating enzymes, α-amylase and α-glucosidase, in the digestive tract. Thus, we considered the study of the proximate composition analysis and α-glucosidase inhibition by the kodo millet extracts to understand the nutritional aspects of the grain which helps in the managing the metabolic disorders.

MATERIALS AND METHODS

Materials

Kodo millet (Paspalum scrobiculatum) was obtained from the super market of Kolar and Tumkur, Karnataka, India. The sample was grinded to powder and the flour was sieved and stored in dry place for further analysis. All methods were carried out in accordance with relevant guidelines.

Chemicals and reagents

The chemicals like acids and inorganic chemicals were purchased from SD fine chemicals of analytical grade, India. Methanol High-Performance Liquid Chromatography (HPLC) grade was obtained from rainbaxy chemicals, India. Glucose oxidase kit for amylglucosidase inhibitory assay Erba glucose kit was procured from Transasia biomedicals Ltd., India.

Proximate composition analysis of kodo millet

Estimation of moisture content: The moisture content of the sample was estimated by Association of Official Analytical Chemists (AOAC) method 930.15, 1990. 0.5 g of flour of sample was transferred to pre-weighed, clean and dry aluminium moisture cups (W1) and recorded the weight (W2). The samples containing aluminium moisture cups were transferred to hot air oven at 105°C for 3 h and transfer to desiccator to cool. After drying transfer the dish to the desiccator to cool. Reweigh the dish and its dried sample (W3). Using the below formula moisture content of the sample was calculated.

\[ \text{Moisture content} = \frac{(W3 - W4)}{W2} \times 100 \]

Estimation of crude protein: Protein content of the sample was determined by the Kjeldahl method as modified by AOAC method 981.10, 1990 method. 0.5 g of the sample was digested by heating with concentrated Sulfuric acid (H2SO4) in a digestion block using (Potassium Sulfate (K2SO4) and Copper(II) Sulfate (CuSO4)) catalyst. Samples were distilled with 10 M Sodium Hydroxide (NaOH). Boric acid (4%) was used to trap ammonia from the distillation and the distillate was titrated with 0.2 M NaOH using mixed indicator. Percent nitrogen was used to estimate percent protein concentration by means of a nitrogen-to-protein conversion factor 6.25. The blank reagent was also titrated similarly.

%Protein = \left( \frac{\text{Vol. Acid} - \text{Vol. Blank}}{1.4007 \times 0.2} \right) \times 6.25 \times \frac{g}{g \ sample}

Estimation of crude fat: The crude fat in the sample was estimated by described in AOAC 920.39, 1990. 5 g flour sample (w) was subjected to drying and the dried sample was transferred to clean and dry extraction thimble, which was packed with glass wool to permit the free flow of solvent. A pre-weighed clean and dry heating flask (w1) was filled with petroleum ether fat was extracted at a condensation rate of 5 to 6 drops per second for 8 hours. After the extraction of fat, the ether was removed by vacuum evaporator and the residual ether was dried in oven at 100°C for 30 min. The flask was then cooled in vacuum desiccator for 2 hours and recorded the weight (w2). Percent crude fat was calculated as:

\[ \frac{(w1 - w2)}{w} \times 100 \]

Estimation crude fibre: The estimation of crude fibre in kodo millet was performed by AOAC method 990.12, 1990. A 2 g of the defatted sample was hydrolyzed by boiling with 150 ml of 0.25 N H2SO4 for 30 min. The suspension was filtered, washed with hot distilled water and the residue was hydrolyzed by boiling with 150 ml of 0.313 N NaOH. The crucible containing sample was dried in oven for 3 hours at 103°C and the weight recorded (w1). The sample was then ignited in a muffle furnace at 600°C for 3 hours and was held in an oven at 103°C for 1 hour and weight recorded (w2). Percent crude fibre was calculated as:

\[ \frac{(w1 - w2)}{w} \times 100 \]

Key Words: Kodo millet, Proximate analysis, α-glucosidase, Diabetes mellitus
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**Estimation of ash:** The crude ash was estimated by AOAC method 942.05, 1990. The 3 g sample was dried in a hot air oven at 100°C for 5 hours and further ignited in a muffle furnace for 3 hours at 600°C. Cool the crucible in desiccators and weigh. At the end of the process the ash content will be in whitish grey color.

\[
\% \text{Crude ash} = \left( \frac{w_2 - w_1}{w_1} \right) \times 100
\]

**Determination of nitrogen free extract:** Nitrogen Free Extract (NFE) or soluble carbohydrates of sample was determined by difference method.

\[
\% \text{NFE} = 100 - (\% \text{moisture} + \% \text{crude protein} + \% \text{crude fat} + \% \text{crude ash} + \% \text{crude fibre})
\]

**Preparation of extracts:** The methanol and aqueous extracts have been used to determine the α-glucosidase inhibitory activity of kodo millet. The 1 g of the millet flour was extracted in methanol (10 ml) for 72 hours at 30°C in rotary shaker. The extract was filtered and the filtrate centrifuged for 15 min and 10000 rpm at 4°C methanol extract was collected. To the above residue was treated with acidified methanol (1% Hydrochloric acid (HCL) in methanol) for 2 hours in a rotary shaker shaking at 80 rpm at 30°C. The extract was cleared by filtration and centrifugation as shown above and was dried using a rotavapor. Then 50 mg of the dried extract was dissolved in 4 ml of distilled water by stirring for 2 hours to get the aqueous extracts heated and without heating for aqueous extracts non-heated. The extract was filtered and centrifuged for 15 min and 10000 rpm at 4°C.

The determination of α-glucosidase activity of test solution was performed using the method with slight modifications. A suitable solution (200 μL) were pre-incubated with α-glucosidase (200 μL in Phosphate (PO₄) buffer) for 15 min at 37°C. To the pre-incubated sample, a 1% starch solution and the volume adjusted to 1 ml by the addition of required amount of phosphate buffer (0.1 M, pH 6.8). The enzyme assay mixture was incubated for 3 minutes and the activity of enzyme was measured by stopped heating the tube in a boiling water bath for 3 minutes. The α-glucosidase inhibition was studied by analysing the release of glucose from starch using standard glucose oxidase/peroxidase enzyme reagent. The released glucose was analysed after 10 minutes of addition of Glucose Oxidase/Peroxidase (GOD/POD) reagent. The absorbance was measured at 510 nm. The solution without a sample (inhibitor) used as a blank. Calculating percentage inhibition of α-glucosidase activity by the following formula using standard curve.

\[
\text{Inhibition of } \alpha \text{-glucosidase} = \left( \frac{\text{Oxidase of Control} - \text{Oxidase of Sample}}{\text{Oxidase of Control}} \right) \times 100
\]

**RESULTS AND DISCUSSION**

**Determination of the proximate analysis**

The results on the proximate composition of kodo millet (Paspalum scrobiculatum) is prescribed in the Table 1. The moisture content of kodo millet was found to be 6.0%. Our results matches with the earlier report by Neelam et al., [2] of 7.3%. Kulkarni et al., [5], Thilagavathi et al., [6], Revathi et al., [7], Kumar et al., [8], Sheela et al., [9], reported the higher moisture content in kodo millet of 10.17%, 11.72%, 9.7%, 12.71% and 11.82% respectively. The present crude protein in the kodo millet was found to be 6.61%. Various other investigations reported the values of 8.30% by Mitkal et al., [10], Patil et al., [11], reported the highest crude protein content in kodo millet of 13.80%. Our investigation showed the lower protein content in kodo millet compare to other previous literature, this is due to the different sample variety and analytical variations.

**TABLE 1**

**Proximate composition of kodo millet**

<table>
<thead>
<tr>
<th>Component</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>6</td>
</tr>
<tr>
<td>Crude protein</td>
<td>6.61</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>8.5</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.82</td>
</tr>
<tr>
<td>Ash</td>
<td>0.62</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>63.62</td>
</tr>
</tbody>
</table>

The crude fat in kodo millet flour sample was estimated to 2.82%. Our result showed the higher value than the previous literature reported by Mitkal et al., [10] as 1.30%, similar value observed by Neelam et al., [2] (1.29%), Deshpande et al., [3] (1.4%), Muthamilarasan et al., [12] (1.4%). The crude fiber in the kodo millet was found to be 8.5%. Our results matches with earlier reports by Mitkal et al., [10] and Neelam et al., [2], Kulkarni et al., [5] reported that highest crude fiber in kodo millet such as 6.33% than the little millet (5.73%), proso millet (5.51%) and barnyard millet (5.35%). The previous studies showed crude fiber of 9.0% by Muthamilarasan et al., [12], 9.2% by Nithyashree et al., [13], 9.6% by Sheela et al., [9], 9.7% by Sharma et al., [14], 9.95% by Thilagavathi et al., [6]. Our investigation showed the carbohydrate content present in the kodo millet was estimated as 75.45%. Similar results are reported by Revathi et al., [7] of 78.0% and Patil et al., [11] reported 71.81%. Other investigations reported the total carbohydrate content in kodo millet as 63.62%, 65.3%, 65.50%, 65.9%, 68.2% and 67.4% by Thilagavathi et al., [6], Muthamilarasan et al., [12], Sheela et al., [9], Rajkumar et al., [15], Nithyashree et al., [13] and Mounika et al., [16]. Hymavathi et al., [17] reported the carbohydrate content present in dehulled kodo millet was 74.71 g. The ash content was found to be 0.62%. Our report falls within the range of earlier reports by Geervani and Eegum [18] and Hymavathi et al., [17] in the dehusked kodo millet grains contains 1.04% and 0.83 g ash content. The sprouted kodo millet showed the ash content of 3.70% [19].

**Amyloglucosidase inhibitory activity of kodo millet**

Our results recorded the promising inhibition activity of kodo millet. Figure 1 indicated the inhibition activity of Kodo millet of 15.37% in methanol, 49.3% in acid methanol, 48.6% in acid methanol heated extracts, whereas aqueous extracts heated reported the highest inhibition activity of 50.26% than the aqueous extracts non-heated (12%). Chiranthika et al., [20] reported kodo millet showed the inhibitory activity of 45.5%. Goudar et al., [21] reported the free and bound form of kodo millet showed the α-glucosidase inhibition activity of 92.6% and 34.49%. α-glucosidase inhibitors help in the slow down the carbohydrate digestion hence reduces the blood sugar level [22,23]. Amyloglucosidase is an enzyme which helps in the breakdown the starch into glucose in the digestive system, resulting in the raise of blood glucose level. Hence, it becomes the chosen factor for controlling the diabetes mellitus.

**REFERENCES**

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