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COMPARATIVE STUDY OF NUTRITIONAL AND ANTIDIABETIC PROPERTIES OF KODO AND BROWN TOP MILLETS

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ABSTRACT

Millets are nutrient-dense grains containing a wide range of bioactive compounds associated with various health-promoting properties. This study evaluated the nutrient value, phytochemical constituents, antioxidant capacity, antidiabetic potential, and predicted glycaemic index (pGI) of raw and cooked kodo millet (*Paspalum scrobiculatum L.*) and brown top millet (*Urochloa ramosa*). Brown top millet showed higher protein (13.37%), fat (4.23%), ash (5.48%), and energy (346.39 kcal/100 g), whereas kodo millet contained higher dietary fibre (9.00%) and carbohydrate (65.20%). Raw kodo millet had the highest phenolic content (984.62 mg GAE/100 g) and flavonoid content (465.45 mg QE/100 g), along with the highest DPPH scavenging activity (71.39%) and lowest IC_{50} (34.97 µg/mL). Kodo millet showed stronger α-amylase inhibition (90%), while brown top millet showed greater α-glucosidase inhibition (70%). Raw kodo millet also exhibited the lowest starch hydrolysis (4.81%) and pGI (43.07), compared with 27.45% and 61.25, respectively, for raw browntop millet. Cooking slightly decreased activity but retained substantial functional properties. Overall, both millets showed promising functional and antidiabetic potential, with raw kodo millet demonstrating greater potential for glycaemic management.

KEYWORDS: Kodo millet, Brown top millet, Antidiabetic activity, In vitro glycaemic index, functional foods, diabetes management.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by impaired glucose regulation due to inadequate secretion of insulin (Type 1), reduced response (Type 2) to it, or both. It may also lead to complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy **(WHO, 2024; Galicia-Garcia *et al.*, 2020)**. Although conventional antidiabetic drugs are effective in glycaemic control, their long-term use may be associated with adverse effects and increased economic burden, encouraging interest in safer and affordable natural alternatives **(ADA, 2024; Davies *et al.*, 2022)**.

Millets have attracted considerable attention as functional foods because of their dietary fibre, resistant starch, low glycaemic index, and diverse bioactive phytochemicals, which may contribute to improved glucose regulation and reduced oxidative stress **(Anitha *et al.*, 2021)**. *Paspalum scrobiculatum L.* (kodo millet) and *Urochloa ramosa* (browntop millet) are relatively underutilized grains containing flavonoids, phenols and other health-promoting bioactive compounds **(Nithiyanantham *et al.*, 2019)**.

The present study investigated the proximate and phytochemical composition, antioxidant activity, in vitro antidiabetic potential, and predicted glycaemic index of raw and cooked kodo and browntop millet's ethanolic extracts.

2. MATERIAL AND METHODS

2.1 Sample Collection and Extraction

Kodo (*Paspalum scrobiculatum L.*) and Brown top (*Urochloa ramosa*) millets were procured from a local organic market in Bengaluru, Karnataka, India (Fig1). Raw and cooked samples of both millets were dried under shade and milled into a fine powder. The extraction was then performed by cold maceration using ethanol at a sample-to-solvent ratio of 1:10 (w/v). The mixtures were maintained under refrigerated conditions (4 °C) for 24 hours with intermittent shaking and subsequently centrifuged over a period of 10 min at 5,000 rpm. The resulting supernatants were collected and maintained at 4 °C until further analysis.

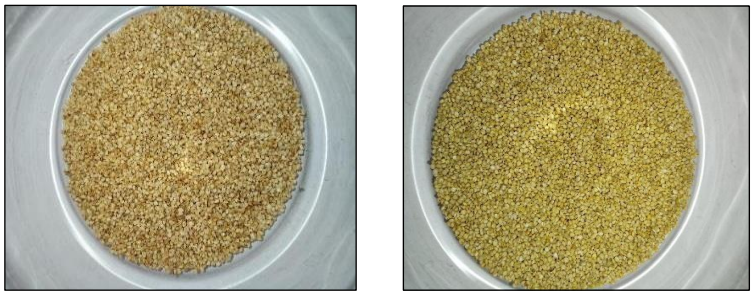


Fig. 1: Kodo (*Paspalum scrobiculatum L.*) and Brown top (*Urochloa ramosa*) millets.

2.2 Proximate and Phytochemical Analysis

The protein, fat, moisture, crude fiber, ash, energy and carbohydrate levels were evaluated from the samples according to established AOAC protocols **(AOAC, 2016)**. Furthermore, the extracts were qualitatively screened for the presence of different phytochemical groups, such as tannins, phenols, flavonoids, glycosides, alkaloids, reducing sugars, carbohydrates, and triterpenoids.

2.3 Determination of the Total Phenolic and Flavonoid Content (TPC and TFC)

The Folin–Ciocalteu assay was performed to determine total phenolic content (TPC), using gallic acid (20–100 µg/mL) as the standard, with absorbance measured at 700 nm **(**Waterhouse, A. L. 2002**)**. Total flavonoid content (TFC) was evaluated via the aluminium chloride method, utilizing quercetin (20–100 µg/mL) as the standard, and absorbance at 450 nm was measured after 30 minutes incubation **(Chang *et al.*, 2002)**. The final TPC and TFC values were reported as mg GAE/100 g and QE/100 g of extract, respectively.

2.4 Free Radical Scavenging Assay (DPPH)

The DPPH method was performed to determine antioxidant activity (Viswanath, V.et al. 2009).The antioxidant potential of the millets were assessed by evaluating their potential to scavenge DPPH radicals. Different concentrations (20–100 µg/mL) of the millet samples combined following the addition of a freshly prepared DPPH reagent and maintained under ambient conditions in the absence of light for 45 min, the absorbance at 517 nm was measured. Ascorbic acid was employed as the standard for the assessment of Antioxidant activity. The DPPH radicals scavenging activity inhibition was calculated and IC_{50} values were determined in relation to the antioxidant potential exhibited across different extract concentrations.

2.5 Antidiabetic Activity

The α-amylase and α-glucosidase inhibition assays were employed to evaluate antidiabetic activity. For α-amylase activity, millet extracts (20-100 µg/mL) were pre-incubated with the starch substrate and enzyme (Narkhede et al. 2011) with slight modifications. After incubation, the reaction was terminated by cooling on an ice bath, followed by measuring at 540nm. The α-glucosidase inhibition was assessed by pre-incubating the extracts (20-100 µg/mL) with α-glucosidase in phosphate buffer pH 6.8 (0.1 M), adding pNPG substrate, and determining absorbance at 405 nm was measured. Sodium carbonate (0.1 M) used to terminate the reaction. Acarbose (20–100 µg/mL) acted as the positive control, and results were calculated as percent inhibition of the enzyme.

2.6 Estimation of Predicted Glycemic Index (pGI)

INFOGEST protocol (Brodkorb et al., 2019) with slight modifications, five grams millet flour was digested using simulated salivary fluid (SSF) (Preparation of the stimulated fluids given in the table 1) which contained α-amylase (0.5 mg/mL) were maintained under refrigerated conditions (37 °C) and 30 s incubation followed by SGF (simulated gastric fluid) containing pepsin (1 mg/mL) at pH 1.9 with 2 hrs incubation.

Table 1: Chemical compositions of Stimulated solution for in vitro digestion.

Sl.no	COMPONENT	MOLARITY	SSF (mL)	SGF (mL)	SIF (mL)
1	HCL	6	0.18	2.6	1.4
2	KCL	0.5	30.2	13.8	13.6
3	NaCl	2	-	23.6	19.2
4	$NaHCO_3$	1	13.6	25.0	85
5	KH_2PO_4	0.5	30.2	13.8	13.6
6	$MgCL_2(H2O)_6$	0.15	1.0	0.8	2.2
7	$(NH_4)_2CO_3$	0.5	0.12	1.0	-

The intestinal stage of digestion was simulated using SIF (simulated intestinal fluid) containing pancreatin (4 mg/mL) at pH 7.0 and 37 °C. Aliquot samples were taken at 30, 60 and 90 min intervals. The Reducing sugars were measured with the DNS reagent. The value of H90 was calculated to estimate the pGI by using the equation: pGI = 39.21 + 0.803(H90).

3. RESULTS AND DISCUSSION

3.1 Sample Extractions

Kodo millet (*Paspalum scrobiculatum L.*) and browntop millet (*Urochloa ramosa*) samples were extracted with ethanol and subsequently used for phytochemical, antioxidant, and antidiabetic analyses. The extracts were designated as RKEE (Raw Kodo millet Ethanolic Extract), CKEE (Cooked Kodo millet Ethanolic Extract), RBEE (Raw Browntop millet Ethanolic Extract), and CBEE (Cooked Browntop millet Ethanolic Extract).

3.2 Proximate Composition

Table 2 shows the proximate composition of Kodo and browntop millet. Both millet varieties showed distinct nutritional profiles. Browntop millet had higher protein (13.37%), fat (4.23%), ash (5.48%), and energy (346.39 kcal/100 g), whereas kodo millet contained more dietary fibre (9.00%), carbohydrate (65.20%), and moisture (11.37%). The kodo millet content high amount of fibre 9.0% which increased satiety and help moderate postprandial glucose levels, while the brown top millet content greater protein (13.37%) and minerals were reported to provide potential benefits for nutrition. Millets were recognized as a good source of food with functional and nutritional values (Saleh et al., 2013).

Table 2: Proximate analysis of kodo millet and brown top millet.

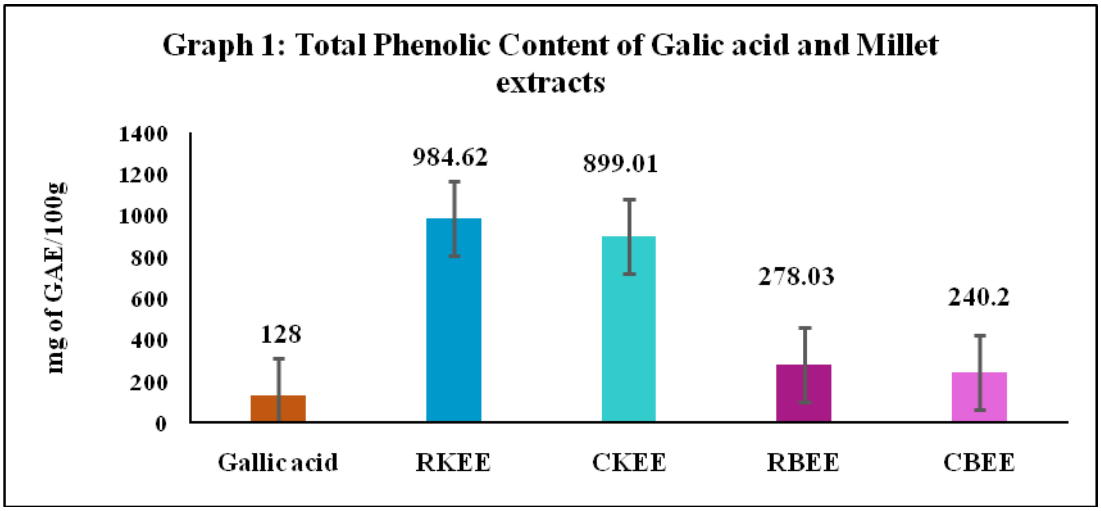
Samples	Moisture %	Protein %	Fat %	Crude Fibre %	Ash %	Carbohydrate %	Energy (Kcal/100g)
Kodo millet	11.37 ±0.07	10.44 ±0.04	1.39±0.08	9.0±0.03	2.6± 0.19	65.2± 0.21	315.07 ±0.28
Brown top millet	8.29 ±0.04	13.37±0.05	4.23±0.03	4.92±0.05	5.48 ±0.06	63.81 ±0.17	346.39 ±0.57

3.3 Qualitative Screening of Phytochemicals

Kodo and brown top millet extracts contained phytochemical compounds with phenols and flavonoids that reacted intensely (+++) with the extract and moderately (++) with cooked extract indicating partial degradation upon heating. Tannins were moderately present while alkaloids and terpenoids were absent. Glycosides were more abundant in kodo millet. Overall, the phytochemicals were more abundant in RKEE than in the other samples. Millets and food grains extracts are a solid source of antioxidants (Chandrasekara & Shahidi, 2012; Shahidi &Ambigaipalan, 2015).

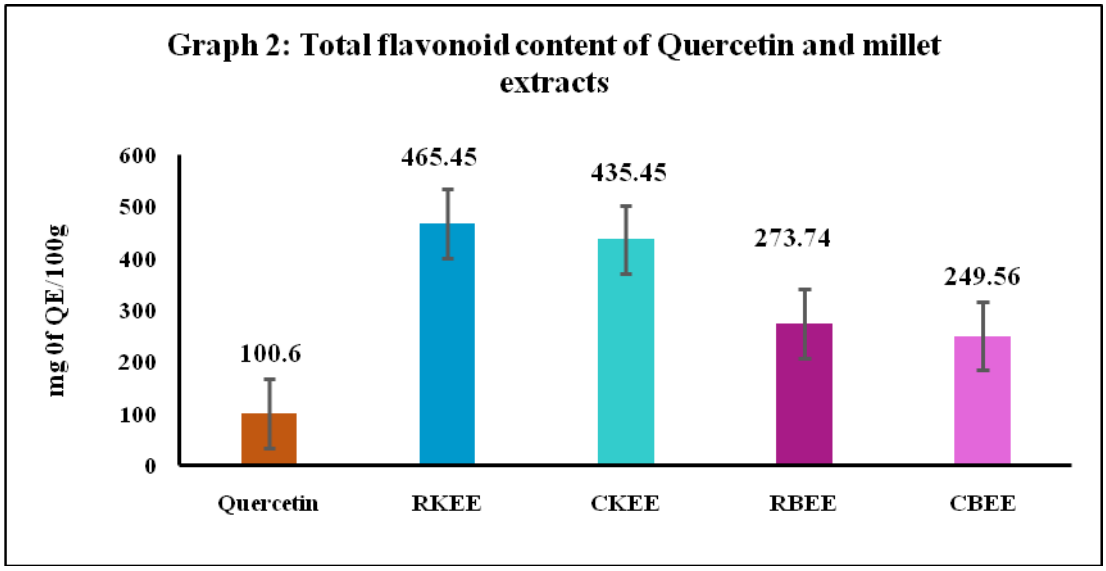
3.4 Determination of Total Phenolic Content

The total phenolic content was determined as mg gallic acid equivalents (GAE) per gram using the equation obtained from a standard gallic acid curve and $y = 1.255x - 2.3$ ($R^2 = 0.9795$). The TPC increased with extract concentration in all samples. RKEE showed the highest values (218.79–984.62 mg GAE/100 g), followed by CKEE (208.87–899.01), RBEE (81.00–278.03), and CBEE (57.50–240.20) (Graph1). Lower phenolic levels after cooking may result from thermal degradation and leaching, although cooked samples retained considerable phenolic content (Hithamani& Srinivasan, 2014).



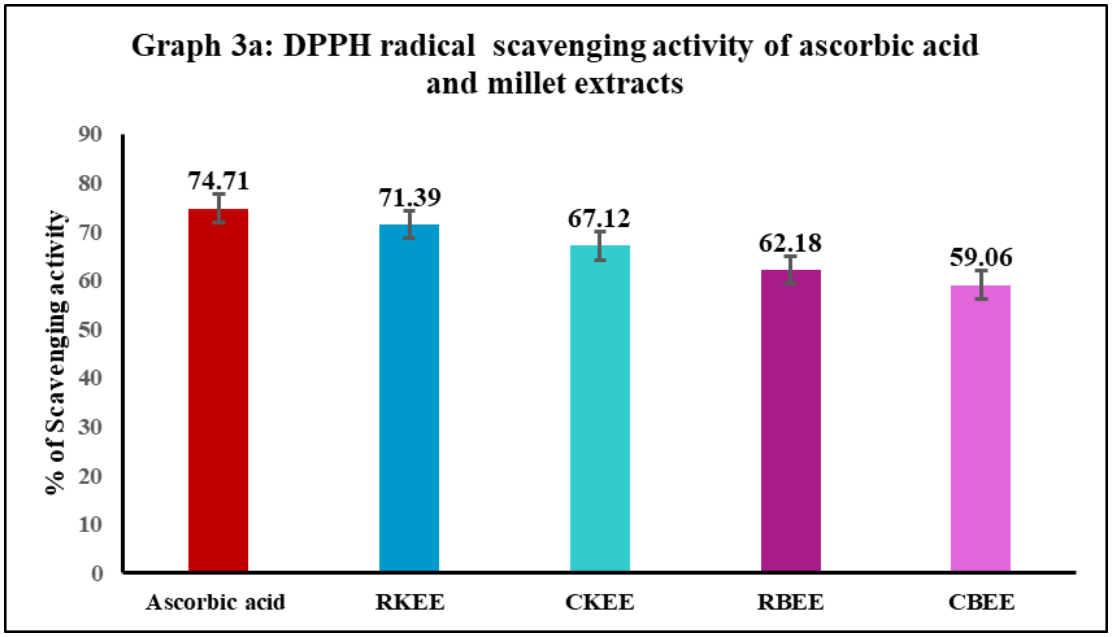
3.5 Determination of Total Flavonoid Content

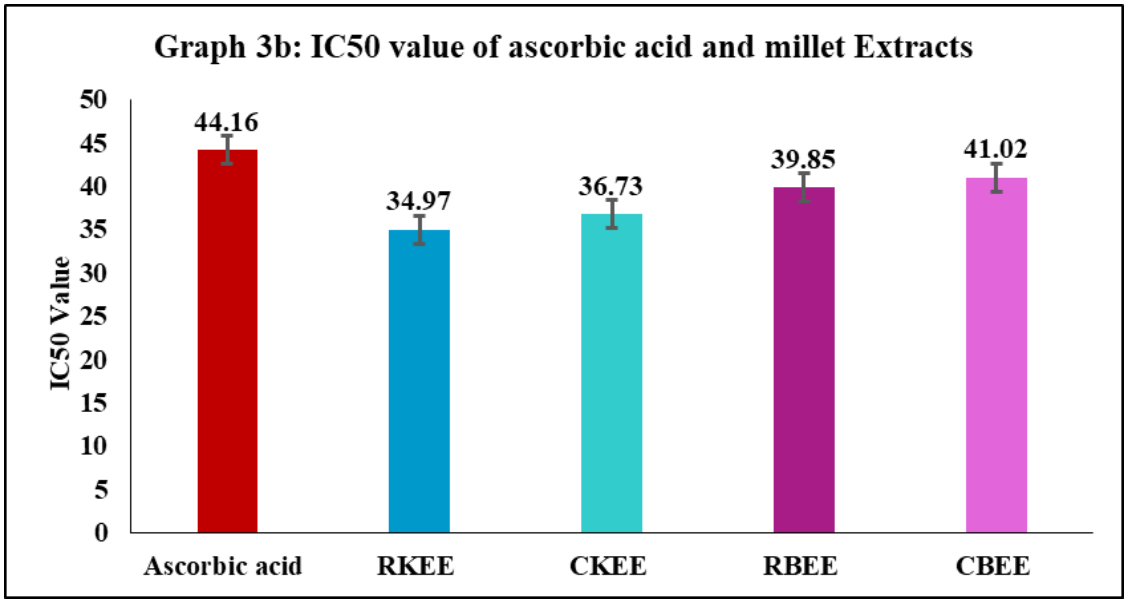
The total flavonoid content was calculated from the quercetin standard calibration curve ($y = 0.9324x + 6.0171$ ($R^2 = 0.9761$). The TFC increased with concentration of extract, with RKEE showing the highest values (98.30–465.45 mg QE/100 g), followed by CKEE (93.36–435.45), RBEE (62.98–273.74), and CBEE (58.78–249.56) (Graph 2). The quercetin calibration curve was linear ($R^2 > 0.99$). Cooking caused a moderate reduction in flavonoid content, while raw kodo millet retained the highest levels, supporting its potential antioxidant activity (Pietta, 2000).



3.6 Radical Scavenging Activity (DPPH)

The scavenging activity increased with extract concentration (20–100 µg/mL) with the highest activity exhibited in the RKEE (30.74–71.39%), followed by CKEE (27.04–67.12%), RBEE (23.08–62.18%), and CBEE (20.54–59.06%) (Graph3a). The linearity was good ($R^2 = 0.9290–0.9898$), although ascorbic acid showed greater activity. The similarity in ranking to TPC and TFC suggests that phenolics and flavonoids contributed substantially to radical-scavenging activity of these extracts. The higher antioxidant activity may be attributed to the hydrogen- or electron-donating capacity of these compounds (Shahidi & Ambigaipalan, 2015).



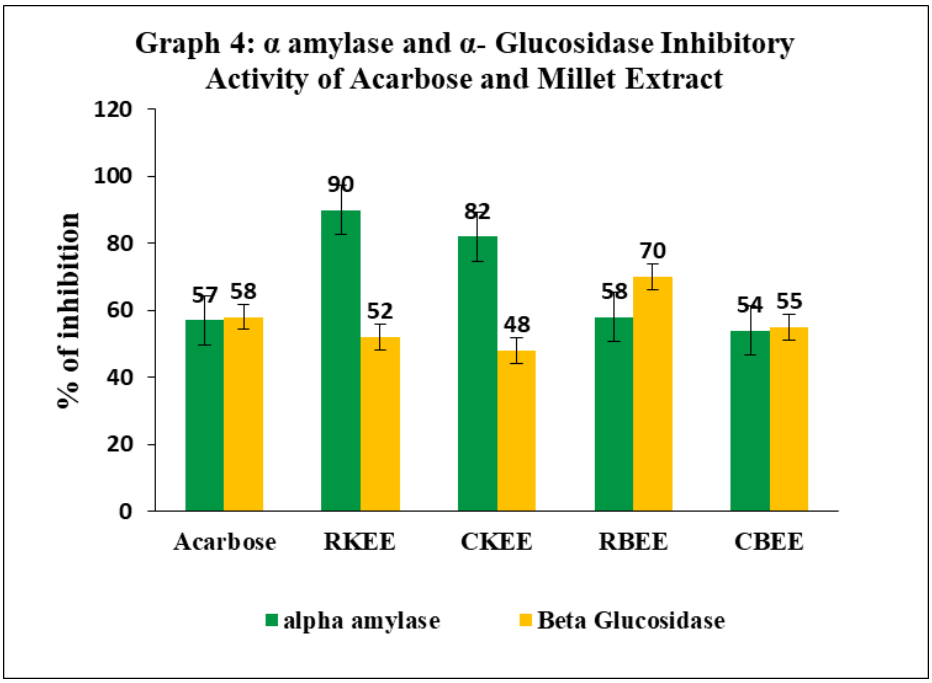


RKEE showed the lowest IC_{50} (34.97 µg/mL), indicating the strongest scavenging activity, followed by CKEE (36.73), RBEE (39.85), and CBEE (41.02 µg/mL) (Graph3b). Cooking slightly increased IC_{50} values, but the extracts retained most of their activity. Overall, raw kodo millet extract demonstrated greater antioxidant potential, likely due to its higher phenolic and flavonoid content (Hithamani& Srinivasan, 2014).

3.7 Anti – diabetic activity

3.7.1 α-Amylase Inhibition Activity

All extracts showed concentration-dependent α-amylase inhibition. RKEE exhibited the highest activity (36–90%), followed by CKEE (28–82%), RBEE (18–58%), and CBEE (15–54%). At 100 µg/mL, RKEE showed greater inhibition than acarbose (57%) (Graph 4). The stronger activity of kodo millet could be related to its higher degree of phenolic and flavonoid content, which can inhibit carbohydrate-hydrolysing enzymes (Sunagar, R. R., & Sreerama, Y. N. 2023; Visvanathan R et al., 2024).

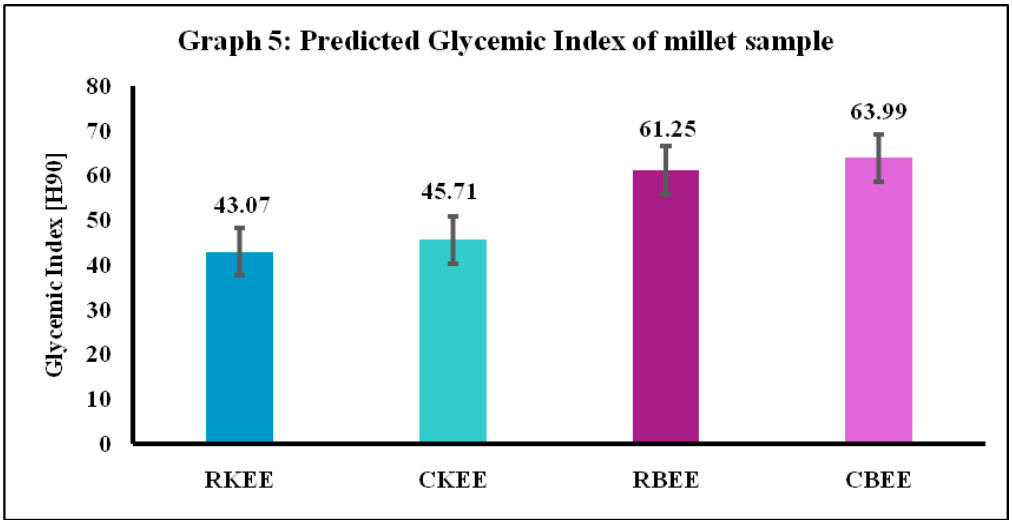


3.7.2 α-Glucosidase Inhibition Activity

In contrast, browntop millet showed stronger α-glucosidase inhibition, with RBEE (24–70%) and CBEE (18–55%), outperforming RKEE (16–52%) and CKEE (13–48%). RBEE also exceeded acarbose (58%) at 100 µg/mL (Graph 4b). These results indicate enzyme-specific distinctions between the two millets and suggest that browntop millet potentially has a greater capacity for α-glucosidase-targeted glucose management. This effect may be related with phenolic and flavonoid component, supporting their potential antidiabetic properties (Rahman S U *et al*., 2024).

3.8 Predicted Glycaemic Index

In vitro digestion showed lower starch hydrolysis in kodo millet than browntop millet. Raw kodo millet had the lowest starch digestion (4.81%) and pGI (43.07), which increased slightly after cooking to 8.10% and 45.71(Graph 5). Browntop millet showed higher values, starch content increasing from (27.45%) and pGI(61.25) in raw millet to (30.86%) and (63.99) in cooked millet. Cooking likely increased starch digestibility through gelatinisation, while kodo millet's higher fibre and resistant starch may have limited digestion **(Annor *et al.*, 2017)**.



4. CONCLUSION

The outcomes of the study speculated that both kodo millet (*Paspalum scrobiculatum L.*) and browntop millet (*Urochloa ramosa*) demonstrated good nutritional and antidiabetic potential. Browntop millet contained higher protein (13.37%), fat (4.23%), ash (5.48%), and energy (346.39 kcal/100 g), whereas kodo millet had higher dietary fibre (9.00%) and carbohydrate (65.20%).

The Raw kodo millet had the greatest level of total phenolic content (218.79–984.62 mg GAE/100 g), flavonoid content (98.30–465.45 mg QE/100 g), and DPPH scavenging activity (30.74–71.39%), with the lowest IC_{50} (34.97 µg/mL). It also showed stronger α-amylase inhibition (36–90%), while browntop millet showed greater α-glucosidase inhibition, with RBEE reaching 70%.

In vitro digestion results further supported kodo millet, had the lowest starch hydrolysis (4.81%) and pGI (43.07) in its raw form, compared with 27.45% hydrolysis and a pGI of 61.25 for raw browntop millet. Overall, raw kodo millet showed greater potential as a functional food for glycaemic management, while browntop millet remains a nutritionally valuable alternative. Further animal and clinical studies are required to confirm these in vitro findings.

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Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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