



Polyphenolic Fractions from Three Millet Types (*Fonio*, *Finger millet*, and *Pearl millet*): their Characterization and Biological Importance

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ABSTRACT

Background: As antioxidant-rich plant foods, cereals can impede lipid and starch breakdown in the human body, are germane to diabetes management.

Objective: We aim to identify newer sources of phytochemicals and health-promoting constituents desirable antidiabetic and antioxidant properties.

Methods: Three millet types i.e. fonio (*Digitaria exilis*), finger millet (*Eleusine coracana*), and pearl millet (*Pennisetum glaucum*) available locally were investigated for antioxidant ability employing these assays i.e. DPPH, ABTS, H₂O₂, antidiabetic ability employing these assays i.e. α -amylase, α -glucosidase and inhibitory property on glycosylation formation. Preliminary characterization tools were employed i.e. UV-Visible spectroscopy (UV-visible) and Fourier-Transform Infrared Spectroscopy (FTIR) for the polyphenolic confirmation.

Results: The absorbance intensity range 325–425 nm confirmed that polyphenolics are present in the three millet types; most of the biological results showed the activities are dose-dependent. Fonio millet extract revealed the highest activity against hemoglobin glycosylation (29.469 \pm 0.399%) which compared favorably with the standard (acarbose) (29.354 \pm 1.607%). Fonio millet extract also showed the best antioxidant activity (significantly higher% inhibition value = 47.909 \pm 3.472) and the pearl millet revealed the least antioxidant activity (significantly lower% inhibition value = 44.910 \pm 3.597) both at a concentration of 500 mg·ml⁻¹, though all the millet extracts showed activity towards this assay better than the standard (19.883 \pm 2.485%). Fonio millet extract displayed a significantly higher percentage inhibition of α -amylase and glucosidase (43.729 \pm 0.410% and 55.835 \pm 2.198%) than finger millet (39.002 \pm 1.604%; 43.971 \pm 5.849%) and pearl millet (33.223 \pm 2.708%; 30.845 \pm 2.841%), respectively.

Conclusion: The polyphenolic extracts from these millet types have therapeutic potentials, which may play significant roles in type 2 diabetes prevention and management, and hence these millets, especially fonio and finger millet, have the potential to be utilized as functional foods.

1. Introduction

Nutrition is gaining popularity nowadays as simple foods are processed in such a way that organoleptic effects take precedence over their nutritional quality. This is the primary explanation for the pervasive issue of obesity and also for the emergence of many nutrition-related diseases currently prevalent. Works of literature have revealed that appropriate dietary behaviors which include abundant and varied fruit consumption, legumes, cereals, and grains can prevent 11–71% of diabetes and its associated deaths (Kulling and Rawel, 2008; Roy et al., 2014; Taverna and Corrado, 2017; Bello et al., 2019a). Nonetheless, not quite so long ago, the compounds in the identified foods that primar-

ily lead to the prevention of some challenging ailments and cures were found (Taverna and Corrado, 2017; Bello et al., 2019b).

Polyphenols are one of such compounds of significant value, and many of these constituents display considerable greater antioxidant activity than vitamins (Hatcher et al., 2008). These polyphenols are a wide group of at least 10,000 complex molecules containing one or more aromatic rings with one or even more compounds hydroxyl units attached to them. They are abundant in most fruits and vegetables, as secondary plant metabolites. Flavonoids and phenolic acids are the most frequently appearing components of dietary polyphenols. These classes of compounds (polyphenols) are typically involved in the defensive system against various types of stress in plants (Oguntoye et al., 2018;

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Aloko et al., 2019). They protect species against reactive oxygen and nitrogen, UV light, pests, plant predators, and pathogens (Aloko et al., 2019).

For millennia, ancient cultures have harnessed their various pharmacological effects to foster and enhance health. In comparison, our understanding hence the perception of their activities has been very limited until recently (Jamar et al., 2017; Bello et al., 2018). Polyphenols were not even treated so long ago as non-core anti-nutrients. Nowadays there are many pieces of evidence from comprehensive research of their antioxidant, anti-inflammatory, and other numerous biological effects in the prevention of specific diseases including cardiovascular diseases, various types of cancer, and diabetes (Sharaf et al., 2013; Eldeen et al., 2011; Costamagna et al., 2016; Olubunmi et al., 2018; Bello et al., 2019a). Polyphenols prevent diseases primarily because of their antioxidant effects. Variation in epigenetic changes, nevertheless, may also have significant impacts. It has been experimentally established that polyphenols not only prevent specific diseases but also affect the spread of disease, inhibit growth and also help in the healing process (Bello et al., 2018a; Bello et al., 2017) Additionally, certain polyphenols function hormonally and have an inhibitory impact on bone resorption. Hence, polyphenols are now the primary focus of diabetic research, as they show possibilities to become excellent agents for the management and cure of different types of diabetes (Lizcano et al., 2010; Locatelli et al., 2011).

The population of adults living with diabetes mellitus globally has been predicted to increase to about 69% by the year 2030 (Dennedy et al., 2015; Shaw et al., 2010). Hyperglycemia in diabetes mellitus usually leads to the generation of reactive oxygen (ROS) that can cause lipid peroxidation and destruction of the cell membrane, which may eventually lead to diabetic complications such as retinopathy, neuropathy, and cardiovascular disease (Aruna Sindhe et al., 2013; Rajaram, 2013) Research has shown that plants containing natural antioxidants like polyphenols, terpenes, and phenolic acids, possess the ability to scavenge free radicals and inhibit the formation of lipid peroxidation, hence preventing β -cell destruction and preventing diabetes-induced ROS formation (Ogundajo and Ashafa, 2017; Kutan Fenercioglu et al., 2010; Aslan et al., 2010). Reduction of hyperglycemia has been regarded as a major strategy in diabetic therapy and can be achieved by inhibiting the two key enzymes (α -amylase and α -glucosidase) involved in carbohydrate metabolism (Bello et al., 2017; Kutan Fenercioglu et al., 2010; Aslan et al., 2010). The study aims to compare the antidiabetic and antioxidative potential of polyphenolic content of these three popularly consumed millets in Nigeria. This research work will provide data that may prove the efficacy of polyphenolic compounds in these millets to be used in the design of novel functional foods with antidiabetic potential.

Fonio millet (*Digitaria exilis*), known as "acha" in Nigeria, is one of the oldest cereals in West Africa. Fonio millet is one of the major millets cultivated in the savannah regions of Africa (semi-arid tropics) and represents somewhere around 0.1 percent of the total major staple and much less than 0.25 percent of Nigeria's total grain production (Irving and Jideani, 1997; Jideani, 1999). Fonio is grown in the northern states of Nigeria but used primarily as rice, puddings, cereal stale bread, and alcoholic drinks for food. Fonio is rich in nutrients, just like other millets, especially cysteine, methionine, and phenylalanine, which are essential amino acids found in millets (Ballogou et al., 2013). Fonio is a cereal that has been shown to have higher contents of some minerals (iron and calcium) than sorghum and wheat. Even this cereal is high in minerals (iron and calcium) relative to wheat and guinea corn. But, subsistence farmers are only the ones that cultivate fonio, it serves more than 95 percent of homes in northern Nigeria, Burkina Faso, Guinea, and Mali as a staple cereal (Jideani and Jideani, 2011). It is considered a more suitable replacement for semolina and other standard diets, and appropriate food for people with diabetes, owing to its high methionine content and low glycaemic index (Jideani, 1999; Ballogou et al., 2013).

Finger millet (*Eleusine coracana*), also known as Ragi, is an essential millet that is widely grown in different regions of Africa and India. After wheat, rice, maize, sorghum, and bajra, it is ranked 6th in production in India (Chethan et al., 2008; Padulosi et al., 2015). *Eleusine coracana* is an annual herbaceous plant, which is abundantly found in the arid and semi-arid regions of Africa and Asia. It is a tetraploid genus that originally evolved from its wild relative *Eleusine africana* but is self-pollinating. The origin of finger millet is believed to be the uplands of Ethiopia and Uganda. This millet type is regarded as one of the cereals with the most protein. The finger millet contains about 2.5–3.5% minerals, 15–20% dietary fiber, 65–75% carbohydrates, 1–2% ether extractives, and 5–8% protein (Thilakarathna and Raizada, 2015; Grovermann et al., 2018). Of most of the millets and cereals, finger millet contains the highest content of calcium (344 mg / 100 g) and potassium (408 mg / 100 g). The cereal contains low-fat content (1.3 percent) and mainly unsaturated fat. 100 g of finger millet has about 336 KCal of energy on average. The millet, conversely, also contains tannins (0.61%), polyphenols, dietary fiber, phytate (0.48%), and trypsin inhibitory factors, that once were viewed as "anti-nutrients" due to their enzyme inhibitory and metal chelating properties, but are now called nutraceuticals. Being anti-glutinous, finger millet is healthy for people with gluten allergy and celiac disease. Finger millet is easy to digest, rich in amino acids, and non-acid forming (Chethan et al., 2008; Padulosi et al., 2015; Grovermann et al., 2018)

Pearl Millet (*Pennisetum glaucum*) is a good source of dietary and health-promoting compounds compared with the main cereals grown. Nevertheless, significant factors restricting its use are the existence of anti-nutritional factors (phytate, tannins, and oxalic acid), which are responsible for poor mineral availability and low sustaining consistency due to higher lipase activity (Padulosi et al., 2015). Many of the wild relatives have adapted from overcoming floods, extreme heat, cold, and drought, thus modifying or establishing resistance to pests and diseases, resulting in less significant crop losses (Debelo et al., 2020). Because of its unusually high nutrient content, the pearl millet is valuable, which contains high amounts of protein, magnesium, B vitamins, zinc, potassium, phosphorus, calcium, and fats. Besides, high levels of dietary fiber are present which contribute much more to the health benefits of this important grain (Shivakumar et al., 2003; Davaiah et al., 2009).

2. Materials and Methods

2.1. Collection

The samples were collected from Bukuru market, Jos South Local Government Area of Plateau State North Central Nigeria in May 2019 and identified by Mal. Muhammad A., a botanist in the Department of Biological Science, Federal University Dutsin-Ma, Katsina. The samples were washed thoroughly and air-dried (spread on polythene bags) under laboratory conditions. Afterward, the dried samples were then pulverized by the use of a wooden mortar and pestle and thereafter stored in a polythene bag pending laboratory analysis.

2.2. Extraction

The pulverized samples were exhaustively extracted with a 50:50 (ethanol-water) solution using the percolation extraction method. 250 g of the different pulverized samples were weighed in duplicate into different extraction bottles as 400 ml of the 50:50 (ethanol-water) solution were added to each of them until they were soaked. The extracts had different initial colours. The extraction was carried out using the percolation extraction method and then filtered into new different sample bottles. The extracts were concentrated using a rotary evaporator (RE 300, Barloworld Scientific Limited). The acquired extracts were thereafter exposed to dry in an evaporating basin for some days.

2.3. Characterization

2.3.1. Ultra-Violet/Visible spectroscopy

The spectra of the polyphenol extracts of the three millet types were determined by Biochrom Libra PCB 1500 UV-vis spectrophotometer.

2.3.2. Fourier transform infrared analyses (FTIR)

The FTIR was done for the polyphenol extracts of the three millet types using the software and equipment described earlier (Bello et al., 2019a).

2.4. Antioxidant activity

2.4.1. 2, 2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay

DPPH is one of the main assays used in assessing the antioxidant activity of plant material employed in this study. The assay was carried out in harmony with the method described by Oguntoye et al. (Oguntoye et al., 2018).

2.4.2. 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity

This method was carried out employing the method of Oguntoye et al. (Oguntoye et al., 2018). Data used for analysis were collected in duplicate.

2.4.3. Hydrogen peroxide scavenging activity

This method was carried out employing the method of Zhao et al. (Zhao et al., 2011). Data used for analysis were collected in duplicate.

2.5. Antidiabetics

2.5.1. α -glucosidase inhibition assay

The α -glucosidase inhibitory ability of the polyphenolic-rich extracts was detected, employing a reported method with slight modification (the pH of phosphate buffer was slightly increased from 6.9 to 7.4) (Zhao et al., 2011).

2.5.2. α -amylase inhibition assay

The α -amylase inhibitory ability of the polyphenolic-rich extracts was detected, employing a reported method with slight modification (the pH of phosphate buffer was slightly increased from 6.9 to 7.4) (Zhao et al., 2011).

2.5.3. Inhibitory activity of advanced glycation end-products (AGEs) formation

The assay was performed by adding 0.6 mg·ml⁻¹ of the solution of hemoglobin, 1 ml of gentamycin (0.2 mg·ml⁻¹), in a 0.01 M phosphate buffer (pH 7.4), and 20 mg·ml⁻¹ concentration of glucose for 72 h. The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of hemoglobin was measured colorimetrically at 443 nm. The rate of absorption with control was considered as 100% glycosylation (Benzie and Strain, 1996; Harris et al., 2014). The study was carried out in triplicate. The glycosylation of hemoglobin percentage was estimated employing the equation below:

$$\text{Percentage of hemoglobin glycosylation} = (A-B)/C \times 100 \%$$

Where A was the absorbance in the presence of the extracts or polyphenols without glucose, B was the absorbance of the extracts or polyphenols in the presence of the glucose and C was the absorbance of the control (Lu et al., 2019; Mohammadi-Motlagh et al., 2011)

2.6. Statistical analysis

The differences in mean values between different millet phenolic fractions were calculated using a one-way variance analysis (ANOVA) followed by Tukey's test at the level of significance $P < 0.05$. Results were reported as mean \pm standard deviation (SD).

3. Results

3.1. UV spectral analysis

The UV absorbance spectra of 50:50 ethanol-water extracts of the samples were recorded in the range of 250–800 nm. The UV absorbance spectra of the three samples had different spectral shapes and intensities. Various subclasses of polyphenol have very distinct chromophores in their structures. In addition, UV spectra acquired are particularly useful in the first characterization phase aimed at classifying, firstly the class of compound and probably the sub-groups of polyphenols. This is "easily" conceivable in most situations and a more thorough look at the UV spectrum may reveal details about the potential structural features within the subgroup to recognize the type of constituents present and confirm whether polyphenols are present i.e. π -bonds, chromophores, and aromatic rings. For most polyphenols, the UV/Vis spectra exhibit at least two absorption bands, one (Band I) ranging from 240 to 280 nm and another (Band II) from 300 to 450 nm (Harborne et al., 1975). The chemical structure influences the absorption wavelength. (i.e., the number and position of substituents, conjugation degree). As shown in Fig. 1, the UV spectra of the three millet types showed an absorbance intensity in the range of 325–425 nm.

3.2. FTIR spectral analysis

The FTIR spectra of each of the polyphenolic extracts of each millet type were shown in Fig. 2 and Table 1: spectrum A related to polyphenol extract of finger millet, spectrum B related to polyphenol extract of fonio millet, and spectrum C to polyphenol extract of pearl millet. The FTIR spectrum of each of these samples was employed to know the active constituents by their functional groups present in the extract based on the peak values in both functional and fingerprint regions of IR.

3.3. α -amylase

The percentage inhibitory of polyphenolic-rich extracts of pearl, finger, and fonio millets on α -amylase was investigated using acarbose as standard, as shown in Fig. 3 and Table 2. The results showed that the activity was dose-dependent. At various concentrations i.e. 100 μ g·ml⁻¹, 200 μ g·ml⁻¹, 500 μ g·ml⁻¹, these extracts gave inhibitory activity range from 1.313 \pm 2.915% to 43.729 \pm 0.410%, and fonio millet was the most effective. Table 2 showed antidiabetic activity with percentage inhibition values of these millet types using α -amylase assay. Overall, fonio millet revealed the best anti-diabetic properties (significantly lower% inhibition value = 43.729 \pm 0.410; $P < 0.05$) and the pearl millet revealed a poor anti-diabetic activity (significantly lower% inhibition value = 33.223 \pm 2.708; $P < 0.05$) both at a concentration of 500 mg·ml⁻¹ as against 70.065 \pm 1.371% for the standard.

3.4. α -glucosidase

The percentage inhibitory activity of flavonoid-rich extracts of pearl, finger, and fonio millets on α -glucosidase was investigated using acarbose as standard as shown in Fig. 3 and Table 2. The results showed that the activity was dose-dependent. At various concentrations i.e. 100 μ g·ml⁻¹, 200 μ g·ml⁻¹, 500 μ g·ml⁻¹, these extracts gave inhibitory activity range from 2.318 \pm 4.449% to 55.835 \pm 2.198%, and fonio millet was the most effective. Table 3 showed antidiabetic activity with percentage inhibition values of these millet types using the α -glucosidase assay. Overall, fonio millet revealed the best anti-diabetic properties (significantly lower% inhibition value = 55.835 \pm 2.198; $P < 0.05$) and the pearl millet revealed a poor antidiabetic activity (significantly lower% inhibition value = 30.845 \pm 2.841; $P < 0.05$) both at a concentration of 500 mg·ml⁻¹ as against 67.690 \pm 1.412 for the standard.

UV-Spectra of Three Millet-types Polyphenol-Rich Extracts

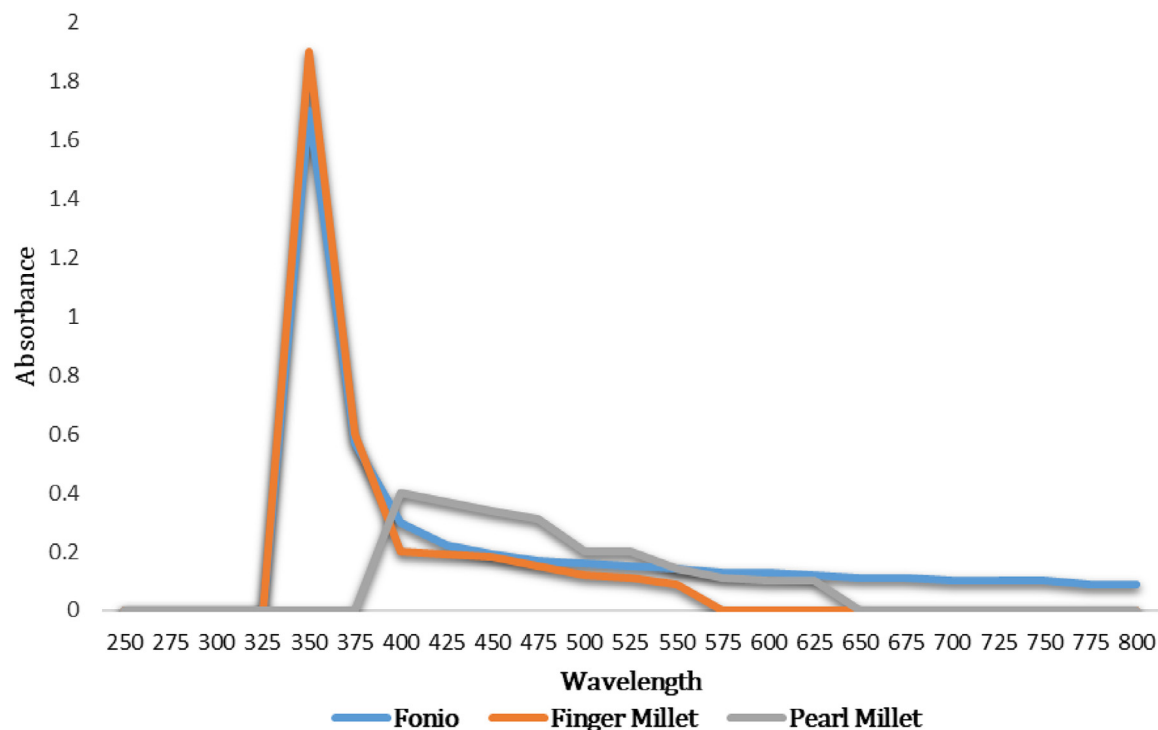


Fig. 1. UV Spectra of three millet types.

Table 1

Recognized FTIR bands in the spectra of the studied samples.

	Finger millet (<i>Eleusine coracana</i>) cm^{-1}	Fonio (<i>Digitaria exilis</i>) cm^{-1}	Pearl millet (<i>Pennisetum glaucum</i>) cm^{-1}
1	3279, 3021	3279, 3017	3286
2	2921	3007	2117
3	2851	2921	1640
4	2087	2854	1462
5	1640, 1628	1711	1112
6	1544	1652	1045
7	1410, 1448	1544	
8	1153	1458	
9	1022	1264	
10	702	1058	

Table 2

Anti-diabetics Activity of the Three Millet-Types (α -Amylase Assay, α -Glucosidase Assay, Inhibition of Glycosylation Assay).

Doses $\mu\text{g}\cdot\text{ml}^{-1}$	α -Amylase Assay				α -Glucosidase Assay				Inhibition of Glycosylation Assay			
	PM	FIM	FOM	ACA	PM	FIM	FOM	ACA	PM	FIM	FOM	ACA
100	1.3 \pm 2.92 ^a	5.1 \pm 2.1 ^a	8.9 \pm 0.7 ^{ab}	16.2 \pm 4.7 ^b	3.9 \pm 0.4 ^a	2.4 \pm 4.5 ^a	14.6 \pm 4.4 ^b	39.6 \pm 1.7 ^c	3.5 \pm 2.3 ^a	0.1 \pm 4.6 ^{ab}	12.6 \pm 4.5 ^b	20.8 \pm 1.7 ^c
200	7.2 \pm 7.1 ^a	6.9 \pm 0.6 ^a	16.5 \pm 6.4 ^{ab}	26.9 \pm 8.9 ^b	16.1 \pm 3.7 ^a	12.4 \pm 4.4 ^a	26.8 \pm 1.8 ^b	40.9 \pm 1.2 ^c	14.2 \pm 3.7 ^a	10.2 \pm 4.5 ^a	25.2 \pm 1.8 ^b	23.1 \pm 0.6 ^b
300	20.2 \pm 2.5 ^a	20.9 \pm 2.2 ^a	28.1 \pm 1.8 ^{ab}	36.5 \pm 8.4 ^b	17.1 \pm 2.1 ^a	27.3 \pm 3.1 ^b	42.6 \pm 5.7 ^c	44.3 \pm 2.3 ^c	15.6 \pm 2.3 ^a	17.3 \pm 0.8 ^{ab}	24.6 \pm 6.3 ^b	25.1 \pm 1.1 ^b
400	24.3 \pm 2.3 ^a	27.2 \pm 1.9 ^a	39.2 \pm 0.5 ^b	48.1 \pm 1.9 ^b	24.5 \pm 4.4 ^a	39.7 \pm 2.3 ^b	49.108 \pm 1.1 ^c	67.7 \pm 1.4 ^c	17.7 \pm 1.1 ^a	19.3 \pm 2.7 ^a	27.8 \pm 2.2 ^b	27.8 \pm 1.1 ^b
500	33.3 \pm 2.71 ^a	39.1 \pm 1.6 ^b	43.8 \pm 0.4 ^c	70.1 \pm 1.4 ^c	30.9 \pm 2.8 ^a	43.9 \pm 5.9 ^b	55.8 \pm 2.2 ^a	67.7 \pm 1.4 ^d	19.5 \pm 2.2 ^a	21.8 \pm 1.7 ^a	29.5 \pm 0.4 ^b	29.4 \pm 1.6 ^b

PM: Pearl Millet, FIM: Finger Millet, FOM: Fonio Millet, ACA: Acarbose. Results are expressed as mean \pm SD values of triplicate determinations. Different alphabets along a column indicate a significant difference (Tukey's-HSD multiple range post hoc tests, $P < 0.05$).

3.5. Inhibition of haemoglobin glycosylation

The percentage inhibitory activity of flavonoid-rich extracts of pearl, finger, and fonio millets on glycosylation formation, was evaluated employing a positive standard gallic acid as shown in Fig. 3 and Table 2. The results showed that the activity was dose-dependent. At vari-

ous concentrations i.e. 100 $\mu\text{g}\cdot\text{ml}^{-1}$, 200 $\mu\text{g}\cdot\text{ml}^{-1}$, 500 $\mu\text{g}\cdot\text{ml}^{-1}$, these extracts gave inhibitory activity range from 0.057 \pm 4.594% to 29.469 \pm 0.399%. The results showed that fonio millet was the most effective against hemoglobin glycosylation. Table 2 showed the haemoglobin glycosylation inhibitory property with percentage inhibition values of these millet types using an appropriate assay. Over-

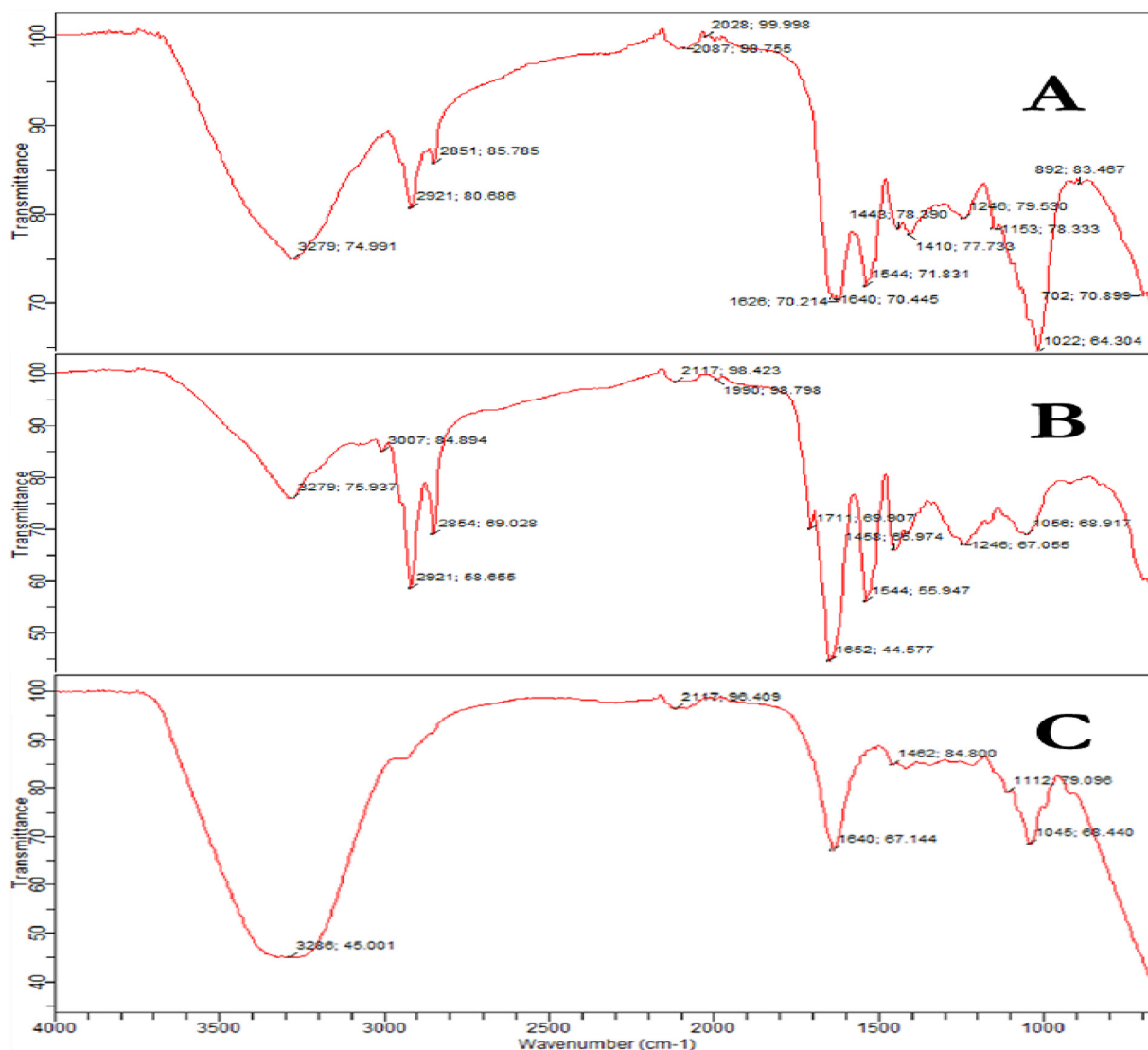


Fig. 2. FTIR Spectra of (A) Finger, (B) Fonio, (C) Pearl millets.

Table 3
Antioxidant Activity of the Three Millet-Types (DPPH Assay, Hydrogen Peroxide Assay, ABTS Assay).

Doses $\mu\text{g}\cdot\text{ml}^{-1}$	DPPH Assay				Hydrogen Peroxide Assay				ABTS Assay			
	PM	FIM	FOM	VIT C	PM	FIM	FOM	VIT C	PM	FIM	FOM	VIT C
10	19.6 \pm 0.2 ^a	19.7 \pm 0.4 ^a	19.2 \pm 0.9 ^a	36.6 \pm 4.8 ^b	9.6 \pm 4.9 ^a	16.1 \pm 1.1 ^a	26.5 \pm 1.1 ^a	13.2 \pm 2.5 ^a	15.6 \pm 0.8 ^a	14.7 \pm 2.1 ^a	14.9 \pm 2.2 ^a	29.4 \pm 4.1 ^b
20	20.2 \pm 0.9 ^a	18.6 \pm 1.8 ^a	19.6 \pm 0.9 ^a	61.2 \pm 4.4 ^b	13.1 \pm 4.7 ^a	18.9 \pm 4.9 ^a	21.6 \pm 3.8 ^a	15.2 \pm 0.1 ^a	14.9 \pm 1.4 ^a	16.7 \pm 5.1 ^a	20.1 \pm 6.9 ^a	49.7 \pm 3.9 ^b
50	19.8 \pm 1.1 ^a	21.2 \pm 0.7 ^a	20.7 \pm 0.7 ^a	60.9 \pm 1.9 ^b	15.3 \pm 2.3 ^a	22.4 \pm 1.3 ^a	31.6 \pm 3.2 ^b	12.9 \pm 2.8 ^b	16.7 \pm 3.7 ^a	21.1 \pm 4.1 ^a	22.9 \pm 4.4 ^a	60.3 \pm 1.2 ^b
100	19.4 \pm 1.6 ^a	22.2 \pm 1.5 ^a	20.8 \pm 1.2 ^a	93.2 \pm 0.1 ^b	33.9 \pm 2.8 ^a	32.7 \pm 7.7 ^b	39.1 \pm 2.3 ^b	17.3 \pm 2.7 ^b	19.2 \pm 2.5 ^a	18.4 \pm 1.5 ^a	19.5 \pm 5.9 ^a	94.9 \pm 0.6 ^b
150	20.9 \pm 0.9 ^a	21.5 \pm 0.4 ^a	26.6 \pm 1.1 ^a	92.9 \pm 0.6 ^b	44.9 \pm 3.6 ^a	46.8 \pm 4.4 ^b	47.9 \pm 3.5 ^b	19.8 \pm 2.4 ^b	14.6 \pm 1.2 ^a	19.4 \pm 2.1 ^a	17.9 \pm 5.9 ^a	94.9 \pm 0.7 ^b

PM: Pearl Millet, FIM: Finger Millet, FOM: Fonio Millet, VIT C: Vitamin C. Results are expressed as mean \pm SD values of triplicate determinations. Different alphabets along a column indicate a significant difference (Tukey's-HSD multiple range post hoc tests, $P < 0.05$).

all, fonio millet revealed the best inhibitory properties (significantly lower% inhibition value = 29.469 ± 0.399 ; $P < 0.05$) which compared favorably with the standard (29.354 ± 1.607), and the pearl millet revealed a poor inhibitory activity (significantly lower% inhibition value = 19.492 ± 2.114 ; $P < 0.05$), all at the concentration of $500 \text{ mg}\cdot\text{ml}^{-1}$.

3.6. α -Diphenyl- β -picrylhydrazyl (DPPH) assay

The results of DPPH radical scavenging activity were indicated in Fig. 3 and Table 3 as compared with a positive standard of vitamin C.

From Fig. 3 and Table 3, we can deduce that the scavenging effects of these millet types' extracts on DPPH radicals were not so remarkable as compared with the standard. Fonio millet showed the most activity as compared to other millet types while pearl was the least by DPPH radical-scavenging assays. The radical scavenging activity is dose-dependent, as the dose increases so does the inhibition percentage. Overall, fonio millet revealed the best antioxidant properties (significantly lower% inhibition value = 26.607 ± 1.059 ; $P < 0.05$) and the pearl millet revealed a poor antioxidant activity (significantly lower% inhibition value = 20.878 ± 0.915 ; $P < 0.05$) both at a concentration of $500 \text{ mg}\cdot\text{ml}^{-1}$ as against 92.881 ± 0.635 for the standard.

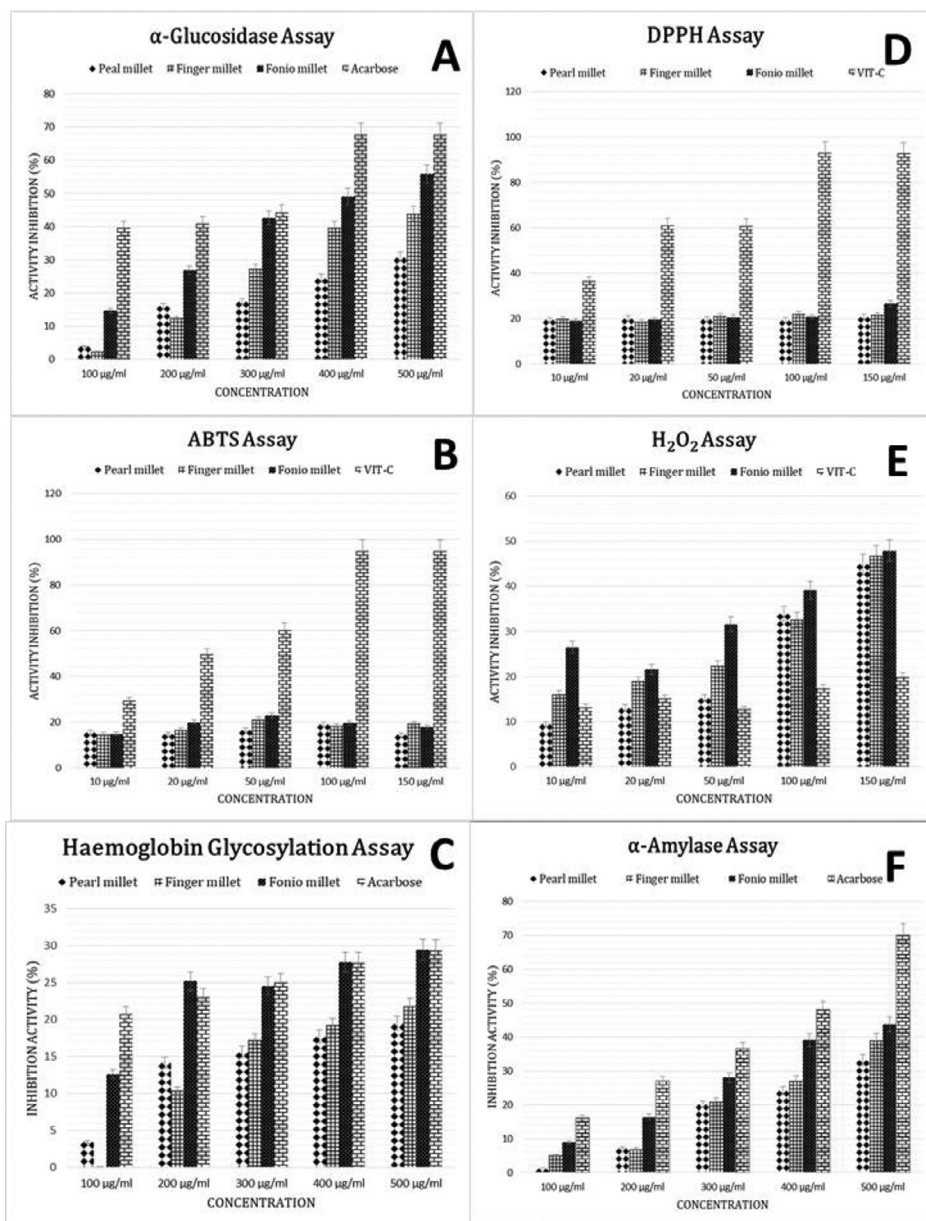


Fig. 3. A: α -Glucosidase Assay of three millet types B: ABTS Assay of three millet types C: Inhibition of Hemoglobin Glycosylation Assay of three millet types D: DPPH Assay of three millet types E: H₂O₂ Assay of three millet types F: α -Amylase Assay of three millet types (All Data are presented as \pm SD of three determinations).

3.7. Hydrogen peroxide assay

The results of H₂O₂ scavenging activity were indicated in Fig. 3 and Table 3 as compared with a positive standard of vitamin C. From Fig. 3 and Table 3, we can deduce that the scavenging effects of these millet types' extracts on lipid hydroperoxides were so remarkable and significant than the standard. Fonio millet showed the best activity as compared to other millet types while pearl was the least by H₂O₂ scavenging assays. The H₂O₂ scavenging activity is dose-dependent, as the dose increases so does the inhibition percentage. Overall, fonio millet revealed the best antioxidant properties (significantly lower% inhibition value = 47.909 ± 3.472 ; $P < 0.05$) and the pearl millet revealed the least antioxidant activity (significantly lower% inhibition value = 44.910 ± 3.597 ; $P < 0.05$) both at a concentration of $500 \text{ mg}\cdot\text{ml}^{-1}$ though all the millet extracts showed activity towards this assay better than the standard ($19.883 \pm 2.485\%$).

3.8. ABTS assay

The results of ABTS scavenging activity are indicated in Fig. 3 and Table 3 as compared with a positive standard of vitamin C. From Fig. 3 and Table 3, we can deduce that the scavenging effects of

these millet types' extracts on ABTS radicals were not so remarkable as compared with the standard. Finger millet showed the most activity as compared to other millet types while pearl was the least by ABTS radical-scavenging assay. The radical scavenging activity is dose-dependent, as the dose increases so does the inhibition percentage. Overall, finger millet revealed the best antioxidant activity (significantly lower% inhibition value = 19.351 ± 2.005 ; $P < 0.05$) and the pearl millet revealed a poor antioxidant activity (significantly lower% inhibition value = 14.571 ± 1.130 ; $P < 0.05$) both at a concentration of $500 \text{ mg}\cdot\text{ml}^{-1}$ as against $94.957 \pm 0.763\%$ for the standard.

4. Discussion

4.1. UV-visible and FTIR characterization

The presence of the major peak at this range showed the presence of polyphenolic compounds, aromatic rings, and π -bonds (Hasana and Desalegn, 2017). The UV spectra displayed unique peaks of polyphenols typical for types of flavonoids and phenolic acids (Pop and Anca Babes, 2015; Sathish, 2012; Kammerer et al., 2004). The functional region, as shown in Fig. 2, has bands at 3286 cm^{-1} , 3274 cm^{-1} , and 3012 cm^{-1} , and these are related to hydroxyl groups ($-\text{OH}$) for all the

millet types. The –OH stretching (3300–2500 cm^{-1}) is the reason for the broad and unique bands spotted, this suggests that there is a likelihood that polyphenols are present. These bands could be associated with stretching (ν) vibration of hydroxyl groups and to –OH attached to the phenolic moiety for the spectrum A, B, and C (Sharaf et al., 2013; Franca et al., 2014). Still on the functional region, in Table 1, 3007 cm^{-1} , 2921 cm^{-1} , 2854 cm^{-1} , 2851 cm^{-1} could be the aliphatic νCH_2 symmetric frequency or may be due to the ethanol. Fingerprint region is within 1600–500 cm^{-1} , there are bands, as shown in Table 1, for spectrum A (1640 cm^{-1} , 1628 cm^{-1} , 1544 cm^{-1}), spectrum B (1711 cm^{-1} , 1652 cm^{-1} , 1544 cm^{-1}), spectrum C (1640 cm^{-1}). These bands may be due to the double bonds (–C = C–), carbonyl bonds (–C = O) groups stretching vibration and may be related to the aromatic ring deformation associated with the polyphenol compounds (Sharaf et al., 2013; Franca et al., 2014; Bello et al., 2020). These bands may also be linked with C = O stretching vibration seen in most polyphenols and their derivatives and maybe symmetric bending vibration of N–H, caffeic acid and its derivatives symmetric bending vibration of N–H (Moç et al., 2011). The bands noticed for all the spectra at 1410 cm^{-1} , 1448 cm^{-1} , 1458 cm^{-1} , 1462 cm^{-1} could be related to CH_2 , CH_3 attached to aromatic rings of polyphenols, the stretching vibration of aromatics and bending (δ) vibration of C–H (Moç et al., 2011; Silva et al., 2014). The vibration of C–O groups of polyols bands, such as hydroxyflavonoids were noticed at 1264 cm^{-1} there was an identified band at 702 cm^{-1} which may be related to aromatic ring vibration or ethanol (Junior et al., 2013; Bello et al., 2020). The FTIR bands at 1022 cm^{-1} , 1045 cm^{-1} , 1058 cm^{-1} , 1112 cm^{-1} may be linked with sugar moiety attached to the polyphenols, they may be related also to the aromatic –OH groups vibration and symmetry stretching of –C–C– (Schulz et al., 2005; Skotti et al., 2014; Rodríguez-Torres et al., 2015). Some authors identified fifty compounds from these millet types employing high-performance liquid chromatography (HPLC), which could be majorly identified as polyphenolic derivatives of both hydroxycinnamic and hydroxybenzoic acids with various classes of flavonoids (Chandrasekara and Shahidi, 2011). A class of compounds (i.e. anthocyanins) was not identified in the polyphenolic extracts of the millet types (finger millet [*Eleusine coracana*], fonio millet [*Digitaria exilis*], pearl millet [*Pennisetum glaucum*]). The authors suggest that anthocyanins may be absent in the millet types. The UV spectra band of this class of compounds (anthocyanins) were absent in Fig. 1 (535 to 544 nm specific to anthocyanins) (Nuryanti et al., 2012). Phenolic acids and flavonoids which belong to polyphenols mostly give these characteristic bands. Phenolic acids such as protocatechuic acid (11), gallic acid (1), *p*-coumaric acid (8), *p*-hydroxybenzoic acid (9), vanillic acid (7), syringic acid (10), ferulic acid (6), vanillin (13) and flavonoids such as quercetin (5), catechin (2), myricetin (12) and epicatechin (3) have been reported in the extracts of these millet types (Fig. 4) (Moç et al., 2011; Chandrasekara and Shahidi, 2011; Barud et al., 2013). Different quantities of these compounds and variations in the compounds' classes lead to a difference in the shape of the FTIR spectra at functional group regions for the millet types

4.2. Antidiabetics activity

Diabetes mellitus is a persistent metabolic malady characterized by hyperglycemia, resulting from the inefficient or deficient secretion of insulin, coupled with changes in protein, carbohydrate, and lipid breakdown (Lebovitz, 2001). Modern literature shows that hyperglycemia can prompt different proteins to non-enzymatic glycosylation and may give rise to the development of serious complications associated with diabetes. Management of postprandial blood glucose increase is indeed important for diabetes care and reduction of chronic vascular complications (Bravo, 1998; Lebovitz, 2001). These could be controlled by highly complex carbohydrate consumption, dietary polyphenols, and a high fiber diet. The millet diet is renowned for its great resilience against diseases in the human body and is commonly recommended for diabetics. A study showed that carbohydrates in various millet types are digested

and absorbed more slowly than those in other cereals (Kavitha, 1995). Regular millet, especially finger and fonio millets, intake is considered to minimize the risk of developing diabetes and digestive disorders, and these attributes have been linked to its high polyphenols and dietary fiber content. The potential benefit of phenolics is attributable to significant inhibition of α -amylase and α -glucosidase during the hydrolysis process of complex carbohydrates and slowing the uptake of carbohydrates i.e. glucose, which eventually regulates the postprandial blood glucose concentrations (Chethan et al., 2008; Misciagna et al., 2000). Polyphenols are known to impede digestive enzymes' activity such as α -amylase, lipases, trypsin, pepsin, and α -glucosidase, and these have been extensively studied. They can act as α -glucosidase and α -amylase inhibitors (comparable to miglitol, voglibose, and acarbose), resulting in reduced postprandial hyperglycemia (CJ, 2001; Rohn et al., 2002). Some authors investigated the antidiabetic and antioxidant activities in vitro of four millet types grown in South Korea. Various assays were employed for antioxidant activity i.e. ABTS, DPPH, and antidiabetics activity i.e. α -glucosidase, α -amylase (Ofosu et al., 2020). Also, finger Italian millet displayed the highest antidiabetic activity with IC_{50} of 18.07 $\mu\text{g}\cdot\text{mL}^{-1}$ inhibition of α -glucosidase and IC_{50} of 10.56 $\mu\text{g}\cdot\text{mL}^{-1}$ when compared with other millets used in the study but significantly lower than the standard acarbose (IC_{50} = 59.34 $\mu\text{g}\cdot\text{mL}^{-1}$ and 27.73 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively) (Ofosu et al., 2020). Some authors investigated the beneficial role of whole-grain flour made from finger (*Eleusine coracana*) and kodo (*Paspalum scrobiculatum*) millets against oxidative stress and control of blood glucose levels in type II diabetes rats (alloxan-induced diabetic rats) over 28 days (Hegde et al., 2005). Cholesterol, lipid peroxides in blood plasma, blood glucose, the tail tendon collagen glycation enzymatic, and non-enzymatic antioxidants were measured. The alloxan-induced diabetic rats which were fed with flour made from kodo millet fortified diet displayed a greater decrease of 27% in cholesterol and of 42% in blood glucose level (42%) than those fed with flour made from finger millet (13% and 36%) respectively. The amounts of enzymatic (vitamins C and E, glutathione) and non-enzymatic antioxidants (glutathione reductase and peroxidase, superoxide dismutase, and catalase) and lipid peroxides in diabetic mice were substantially reduced and recovered to normal levels in the whole-grain flour made from finger (*Eleusine coracana*) and kodo (*Paspalum scrobiculatum*) millets fortified diet groups (Hegde et al., 2005). These millets were reported to have antioxidant and antidiabetic properties (Hegde et al., 2005). The study concluded that these millet types are good in handling type 2 diabetes complications and stress disorders. In the present study, fonio and finger millet are good inhibitors of α -glucosidase and α -amylase, and other authors also confirmed these properties of the millets-diets (Mani et al., 1993; Kurup et al., 1993). Epidemiological reports have shown a lower prevalence of diabetes and its complications in populations whose diets are rich in millets (Saleh et al., 2013). A study discovered the positive effect of finger millet diet in non-insulin-dependent diabetes mellitus (NIDDM) (Lakshmi and Sumathi, 2002). It was discovered that it is more effective than wheat and rice (reduces the glycemic index than the former) (Kurup et al., 1993).

4.3. Inhibition of AGEs formation

Non-enzymatic in vivo reaction occurs between glucose and proteins. This results in glucose-addition products which are covalently attached to protein which are also crosslinked in-between. The unnecessary large build-up of these glucose-addition products, or can also be called advanced glycosylation end products (AGEs), are alleged to play a major role in the acute complications of diabetes (Lakshmi Kumari and Sumathi, 2002; Rojas et al., 2018). Besides the enzymatic glycosylation, there is a type of glycosylation that occurs within the nucleic acid. The build-up of advanced glycation endproducts (AGEs) is greatly associated with the initiation and progress of complications associated with diabetes, this is a primary pathophysiological process (Rojas et al., 2018; Rowan et al., 2018). In recent times, increased attempts and studies have focused on finding compounds and products from natural sources to help

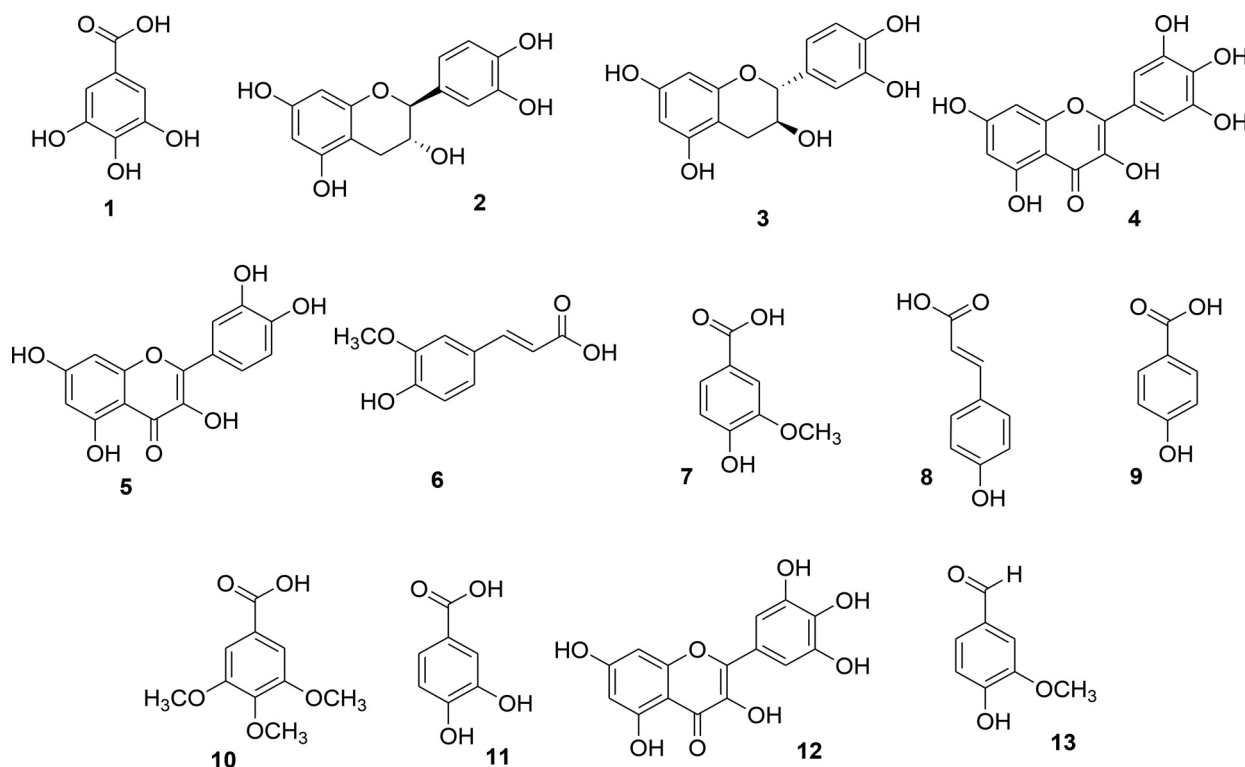


Fig. 4. Identified Compounds from these Millet Types.

stop or reduce the harmful effects of AGEs to the human body, and reports have shown the significant antiglycation activity of polyphenols (Khazei et al., 2016; Yeh et al., 2017; Crasci et al., 2018; Martini et al., 2020; Zhou et al., 2019). The polyphenolic extract from fonio millet displayed the most significant percentage inhibition of AGEs formation value of 29.469 ± 0.399 , which was statistically significant compared to the positive standard employed acarbose with a percentage inhibition value of 29.354 ± 1.607 . Fonio was found to be better than the standard used. Finger and pearl millet extracts showed the percentage inhibition of AGEs formation values of 21.799 ± 1.668 and 19.492 ± 2.114 , respectively. Many authors have affirmed that inhibition of protein glycation by polyphenol intensely relates to their phenolic content and their antioxidant properties. This class of compounds i.e. polyphenols of the medicinal crops and plants may display stronger antiglycation activity than some known antiglycation drugs (Harris et al., 2014; Sekhon-Loodu and Rupasinghe, 2019)

4.4. Antioxidant activity

The value of antioxidant secondary metabolites is increasing because of their unique roles as lipid stabilizing agents and suppressors of unnecessary oxidation initiating aging and cancer (Namiki, 1990). Its stable free radical intermediates prevent the oxidation of different food ingredients, particularly fatty acids, and oils (Cuvelier et al., 1992; Castwilluccio et al., 1995). Antioxidant activity manifests itself in a wide range of actions, such as inhibiting oxidizing enzymes, chelating transition metals, transferring hydrogen or a single electron to radicals, deactivating single oxygen, or enzymatically detoxifying reactive oxygen species. To fully characterize the overall antioxidant activity, different methods should be used to evaluate the antioxidant potential of pure compounds or extracts (Prior et al., 2005; Dykes and Rooney, 2006). The capacity of polyphenols to function as antioxidants stems from their capacity to give hydrogen atoms to electron-deficient free radicals through hydroxyl groups on benzene rings, and then come into being a resonance-stabilized and much less reacting phenoxyl radical (Devi et al., 2014). The ABTS, DPPH, and H_2O_2 are popular procedures

employed to evaluate the antioxidant abilities of secondary metabolites and extracts from natural sources. These methods depend on a spectrophotometer that is able to detect the color changes due to radical saturation. Such methods can therefore display the radical scavenging capability of antioxidants even when existing in intricate biological mixtures such as food extracts. From the results, the three plant polyphenolic extracts were capable of scavenging DPPH, ABTS, and H_2O_2 in a manner that depends on concentration. Therefore, the polyphenolic extracts of fonio (*Digitaria exilis*), finger millet (*Eleusine coracana*), and pearl millet (*Pennisetum glaucum*) as well as the positive control ascorbic acid, were examined about scavenging capacity towards DPPH, peroxides, and ABTS radical cation. Some authors evaluated the phenolic content and the antioxidant effect employing ferrous chelating, β carotene-linoleate, trolox equivalent, and antioxidant capacity (TEAC) assays along with reducing power (RP) of insoluble and soluble-bound phenolic extracts of different types of whole grain millets (kodo, finger, foxtail, proso, pearl, and little millets). High antioxidant properties of such millets were detected by the assays as performed in the present study. This disposition was linked to the varietal differences in phenolic contents of these millet types (Chandrasekara and Shahidi, 2011). The degree of efficacy of antioxidant activity of polyphenolics depends largely on the position and degree of hydroxylation of the phenolic rings, and this activity also depends on the extraction procedure since this type of compound is highly polar (Miyake and Shibamoto, 1997). Several other structural characteristics help to assess the level of antioxidant activity (Bravo, 1998). Some researchers investigated the antioxidant and anti-diabetic properties in vitro of four millet types grown in South Korea. Various assays were employed for antioxidant activity i.e. ABTS, DPPH, and antidiabetic activity i.e. α -glucosidase, α -amylase. The authors discovered that the millets antioxidant activity was assay dependent and that finger Italian millet showed the highest DPPH IC_{50} value of $436.25 \mu\text{g}\cdot\text{mL}^{-1}$ and the highest ABTS IC_{50} value of $381.65 \mu\text{g}\cdot\text{mL}^{-1}$ but these values were generally lower than that of the standard acarbose used. They ascertained that the millet compared favourably with the standard employed (Ofosu et al., 2020). These results complement our study, although the antioxidant capacity of these millet extracts may

not be compared with the standard employed yet their antioxidant activities are significant and assay dependent.

5. Conclusion

Increased nutritional knowledge challenges the food industry in creating new food items with distinctive quality that can improve people's health. These dietary compounds in these millet types i.e. polyphenols have a variety of health benefits such as antimicrobial, antioxidant, antidiabetic, hypocholesterolemic effects, and guard against diet-related diseases especially among the rural populace. This study gives valuable insights into the antidiabetic and antioxidant potential of these millet types which can be gotten locally. Fonio and finger millet extracts displayed high antioxidant ability. The extracts which are major polyphenols exhibited potent inhibition of α -amylase and α -glucosidase activities, they also showed antiglycation activity, showing their ability to lessen the harmful effects of AGEs. The results of this research are important for the development of health-promoting ingredients and functional foods for the prevention and control of diabetes and other chronic diseases.

Ethical Approval

Not applicable.

Data Availability

Nil.

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Declaration of Competing Interest

The authors declare that there is no conflicts of interest among them.

CRedit authorship contribution statement

The BOM, ABO, AB, and MO, played a substantial role in performing experiments, analysis, data acquisition, interpretation, and manuscript preparation. OSO read and edited the original draft. All authors critically revised and finalized the manuscript for publication.

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Supplementary Materials

Nil.

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