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Antioxidant Potential of Crude Powder, Methanol and Aqueous Extracts of Fonio Millet (*Digitaria exilis*) Grains

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Abstract

This study evaluated the antioxidant potential along with total phenolic content, total flavonoid content of the crude powder, methanol and aqueous extracts of *Digitaria exilis* grains. The antioxidant activities of the crude powder and extracts were determined by measuring the reducing ability and hydrogen peroxide scavenging activity. The results showed that methanol extracts exhibited the highest total phenolic content (57.96 ± 6.84 mg gallic acid equivalence/g dried weight) and total flavonoid content (38.75 ± 9.76 mg quercetin equivalence/g dried weight) compared to the crude powder and aqueous extracts. A concentration- dependent increase in the reducing ability and hydrogen peroxide scavenging activity was observed in all the samples. The results were comparable to ascorbic acid, the standard antioxidant used. These results indicate that *Digitaria exilis* grains have antioxidant activity and may account for the use of the grains in traditional systems of medicine.

Keywords: Antioxidant activity; *Digitaria exilis*; Hydrogen peroxide; Reducing ability
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Introduction

Reactive oxygen species (ROS) consist of radical and non-radical molecules such as superoxide anion ($0_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) (Sharma et al., 2012), and are formed during normal cellular metabolism of oxygen or from exposure to xenobiotics. At higher concentrations, they cause oxidative stress by enhancing lipid peroxidation and oxidation of proteins and nucleic acids. Oxidative stress is known to promote the development and progression of atherosclerosis, neurodegenerative disorders, cancer, diabetes and aging (Ray et al., 2012). Therefore, there must be a balance between free radicals and antioxidants in order to maintain normal cellular functions (Lobo et al., 2010). Antioxidants prevent oxidative damage to cells by scavenging the free radicals (Young & Woodside, 2001).

Plant foods, such as fruits, vegetables, legumes and cereals serve as sources of natural antioxidants. They contain bioactive compounds like phenols, flavonoids and other minor components that have significant antioxidant activity. However, numerous studies focused on natural antioxidants obtained from fruits, vegetables and legumes with little emphasis on antioxidant activity of whole grain cereals. Whole grains are used as staple foods and constitute a large amount of diet. Numerous studies have shown that consumption of whole grains protects the body against degenerative disorders and hyperglycemia (Connolly et al., 2012; Durazzo et al., 2015; Dykes & Rooney, 2007).

Fonio Millet (*Digitaria exilis* Stapf) locally known as 'Acha' in Northern Nigeria belongs to the family gramineae and is indigenous to West Africa (Chukwu & Abdul-kadir, 2008). It is cultivated in the upland plateau of central Nigeria and other states like Taraba and Bauchi. Acha grows well in sandy soils and areas with low rain fall. It can grow to a height of 1.4m, the leaves are alternate and simple, and has a glabrous stem. Mature grains are harvested 3-4 months after planting (Vodouche & Achigan-Dako, 2006). The grains are tiny with a light brown seed coat. Acha grains are milled into flour and used in the preparation of local meals and beverages. It has been claimed that the grains have medicinal properties. It is recommended for patients with diabetes and for lactating women, and is also used as weaning food (Vodouche & Achigan-Dako, 2006; Jideani & Jideani, 2011). However, despite the widespread use of *Digitaria exilis* grains in the traditional system of medicine for the management of diabetes, there is very little information on the antioxidant activity of the grains. Thus, the aim of this study is to evaluate the antioxidant potential of the crude powder, methanol and aqueous extracts of *Digitaria exilis* grains.

Materials and methods

Plant Material

Dried grains of Fonio millet were purchased from a local market in Tundu Wada, Zaria, Kaduna state, Nigeria. The grains were ground to powder and stored in a clean container for further analysis.

Extraction Procedure

Thirty kilogram each of the powdered sample was weighed accurately and soaked in 7.5L of distilled water and methanol respectively. The mixtures were left for 72 h at room temperature with mild stirring at regular intervals of 3 h, after which they were then filtered using a Cheese cloth. The solutions obtained were further filtered through a Whatman No 1 filter paper and the filtrates concentrated in a water bath at 60^0C. The semisolid masses obtained were transferred to a desiccator.

Determination of total phenolic content

Using Folin-Ciocalteu reagent, total phenols in the crude powder and extracts were determined according to the procedure reported by Atanassova et al. (2011) with little modification using gallic acid as standard. The crude sample and extracts were made to a concentration of 1mg/mL. One mL of the crude powder or extracts were added to 9 mL distilled deionized water (dd H_2O) in a 25 mL volumetric flask. One mL Folin-Ciocalteu reagent was added, and mixed thoroughly. After 5 min, 10 mL of 7% sodium carbonate was added. The solution was made up to 25 mL with dd H_2O and mixed, after which it was allowed to stand for 90 mins at room temperature. The absorbance was measured at 750 nm against a blank which was made up of ddH_2O only. Standard curve of gallic acid solution (20, 40, 60, 80 and 100 mg/L) was prepared using a similar procedure. The amounts of total phenolic content in the samples were calculated from the calibration curve. The results were expressed as mg gallic acid equivalent per gram (mg GAE/g) dried weight.

The total phenol content was calculated as: $\frac{CV}{M}$

Where C = concentration obtained from the calibration curve, M = mass of the extract used and V = volume of the extract used

Determination of total flavonoid content

Total flavonoids in the crude powder and extracts were determined using aluminum colorimetric assay described by Atanassova et al. (2011) with little modification using quercetin as standard. The crude sample and extracts were made to a final concentration of 1mg/mL with methanol. One mL of the crude sample and extracts were added to 4mL distilled deionized water (dd H_2O) in a 10 mL volumetric flask. 0.3 mL 5% sodium nitrite was also added. After 5 mins, 0.3 mL of 10% aluminum chloride was added. At the sixth minute, 2mL 1M sodium hydroxide was added. The volume was made up to 10 mL with dd H_2O and thoroughly mixed. Absorbance was then measured at 510 nm. Blank solution contained methanol instead of the sample. Standard curve of gallic acid solution (20, 40, 60, 80 and 100 mg/L) was prepared using a similar procedure. The total flavonoid content in each sample was obtained from the calibration curve. The data obtained were expressed as mg quercetin equivalent per gram (mg QE/g) dried weight.

Total flavonoid content was calculated as: $\frac{CV}{M}$

Where C = concentration obtained from the calibration curve, M = mass of the extract used and V = volume of the extract used

Reducing ability

Reducing ability assay was carried out according to the method of Oyaizu (1989). Using ascorbic acid as standard, different concentrations of the crude powder, methanol or aqueous extracts (0.08-0.4 mg/mL) in distilled water (1 mL) was mixed with phosphate buffer (2.5mL) (0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5mL). The mixture was incubated at 50^0C for 20 min and 10% trichloroacetic acid (2.5mL) was added, after which it was centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was then mixed with 2.5mL of distilled water and 0.5 mL 0.1% $FeCl_3$. Absorbance was measured at 700 nm using a UV spectrophotometer.

Hydrogen peroxide free radical scavenging activity

Hydrogen peroxide free radical scavenging activity was determined as reported by Czochra and Widensk (2002) using ascorbic acid as standard. One mL of the crude powder or extracts (20-100 µL [that is 4 mg/ml of plant extracts and crude powder in methanol]) was mixed with 2mL of hydrogen peroxide (43 m mol) and 2.4 mL of 0.1 M phosphate buffer (pH 7.4). The solution was kept for 10 min at room temperature after which the absorbance was measured at 230 nm. The blank solution used contains the plant sample without hydrogen peroxide. The control was prepared without the sample. Hydrogen peroxide free radical scavenging activity was calculated as:

$$[\frac{V_0 - V_1}{V_0}] \times 100$$

Where V_0 = absorbance of control and V_1 = absorbance of sample

Statistical analysis

All experiments were carried out in triplicates and data represented as mean ± standard deviation. Linear regression analysis was done for the total phenolic and total flavonoid content using Microsoft Excel software 2013.

Results

Total Phenolic Content

The total phenolic content (TPC) of the crude powder, methanol and aqueous extract of *Digitaria exilis* grains were determined using the Folin-Ciocalteu method. It was observed that the methanol extract had the highest TPC (57.96 ± 6.68 mg GAE/g) compared to the crude powder (30.86 ± 0.68 mg GAE/g) and aqueous extract (44.98 ± 2.13 mg GAE/g) (Table 1). TPC was calculated from the calibration curve in Figure 1. Where y = 0.0047x + 0.0136 and R^2 = 0.998.

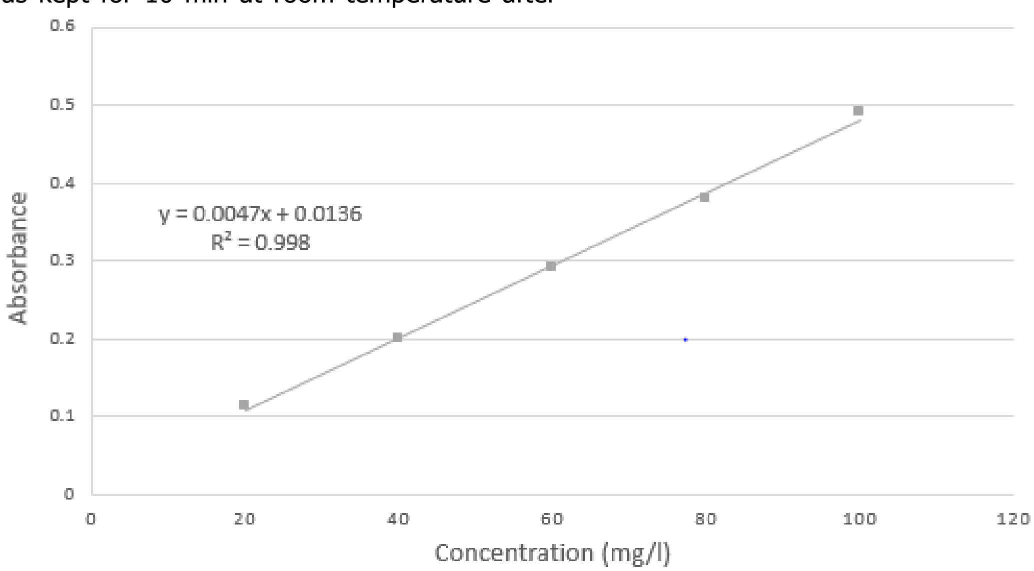

Fig 1: Standard curve of Gallic acid

Table 1: Total phenolic content and total flavonoid content in crude powder, methanol and aqueous extracts of *Digitaria exilis* grains

Sample	Total Phenolic Content[a, c]	Total Flavonoids Content[b,c]
Crude powder	30.86 ± 0.68	16.67 ± 1.90
Methanol extract	57.96 ± 6.84	38.75 ± 9.76
Aqueous extract	44.98 ± 2.13	28.75 ± 5.44

[a] mg gallic acid equivalent/ g dry weight
[b] mg quercetin equivalent/ g dry weight
[c] Data are represented as mean ± standard deviation of replicate measurement (n = 3)

Total Flavonoid Content

As shown in Table 1, methanol extract also had the highest total flavonoid content (TFC) of 38.75 ± 9.76 mg QE/g compared to the crude powder and aqueous extract with TFC of and 16.67 ± 1.90 and 28.75 ± 5.44 mg QE/g respectively. The equation of standard curve (Figure 2) is y = 0.0008x + 0.0005 and the regression coefficient R^2 = 0.9842.

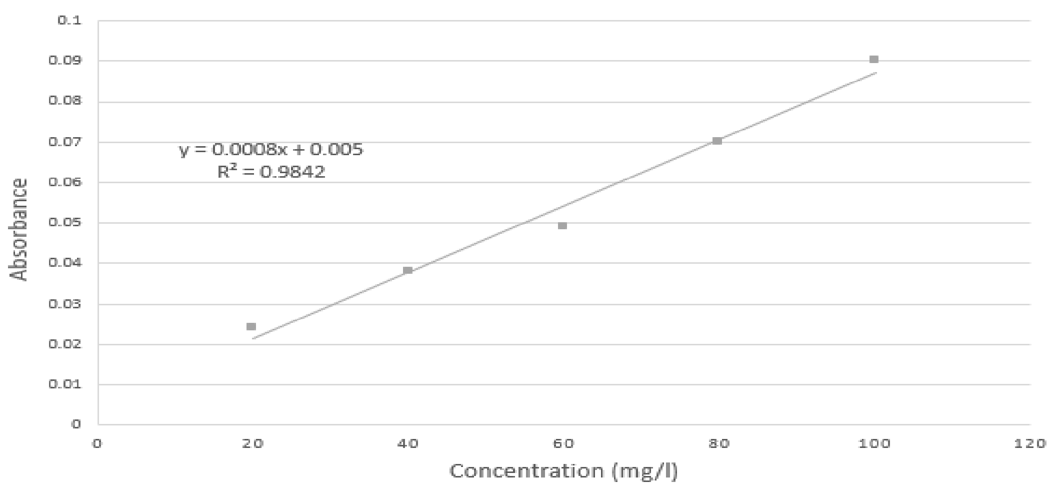

Fig 2: Standard curve of Quercetin

Reducing ability

Antioxidant activities of the crude powder and extracts as determined by the reducing ability are shown in Figure 3. The reducing ability of all the samples increased with increase in the concentrations of the samples. The reducing ability of the samples were compared with the reference standard (ascorbic acid).

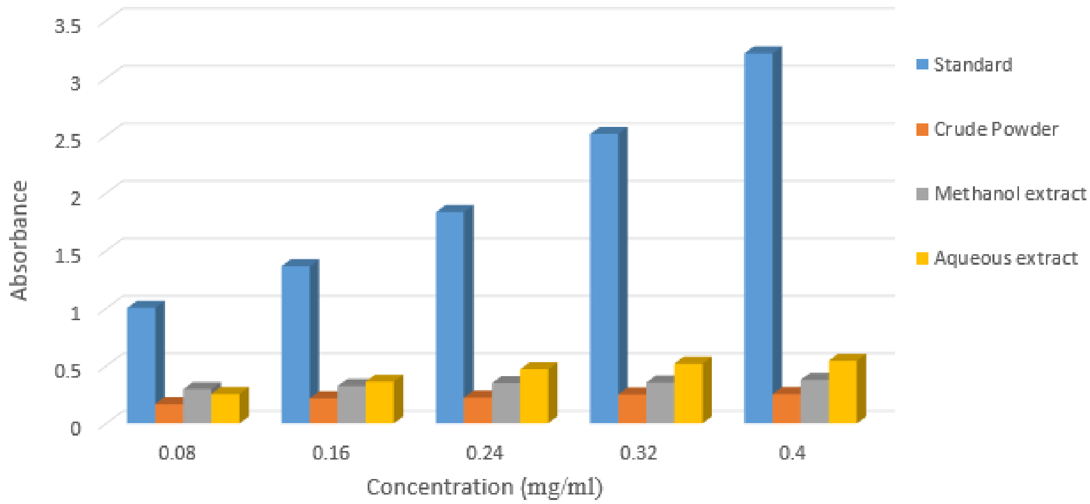

Fig 3: Reducing ability of the crude powder, methanol and aqueous extracts of *Digitaria exilis* grains

Hydrogen peroxide scavenging activity

Figure 4 shows the hydrogen peroxide scavenging activity of the crude powder, methanol and aqueous extracts of Fonio millet. The samples were compared with ascorbic acid standard. The methanol extract showed the highest hydrogen peroxide scavenging activity compared to crude and aqueous extract. However, the hydrogen peroxide scavenging activities of all the samples increased as the concentrations of the samples increased from the initial volume of 20-100µL.

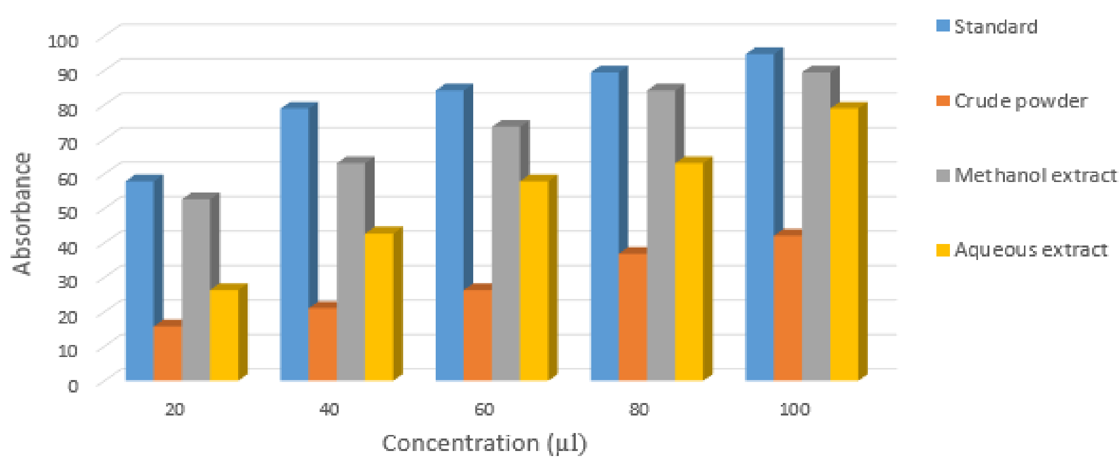

Fig 4: Hydrogen peroxide free radical scavenging activity of crude powder, methanol and aqueous extracts of *Digitaria exilis* grains

Discussion

In this study, the crude powder, methanol and aqueous extract of *Digitaria exilis* grains were analysed for their TPC, TFC and antioxidant potential. Many plant parts contain bioactive substances that are of pharmacological importance. These bioactive components include phenols, flavonoids and alkaloids, many of which have antioxidant and redox properties. Plant phenolics are compounds with aromatic rings having one or more hydroxyl groups which can be simple or complex polyphenols (Balasundram et al., 2006). They are produced by plants in response to environmental stress. Phenolic compounds are good chain breaking antioxidants. They also act as singlet oxygen quenchers, hydrogen donors and reducing agents (Adebiyi et al., 2017). Among the samples analysed, methanol extract had the highest TPC and antioxidant activity.

Flavonoids are a family of polyphenols with low molecular weight. Flavonoids obtained from diet can attenuate hyperglycemia including preventing long term diabetic complications (Taha et al., 2011). Plants with high concentration of polyphenols and/or flavonoids can serve as natural antidiabetic agents because they can reduce oxidative stress, inhibit the production of free radicals and generally serve as antioxidants (Kamtekar et al., 2014). The pharmacological effect of *Digitaria exilis* grains in traditional medicine could be attributed to the presence and high concentration of total phenolics and total flavonoids contents in the grains. The methanol extract also had the highest amount of TFC. The high concentration of TPC and TFC in the methanol extract may be due to the polarity and type of solvent used in extraction (Lin et al., 2016). That's because polyphenols are polar compounds that are easily extracted in polar solvents such as methanol and water (Dabire et al., 2015)

The reductive ability of the crude powder and extracts was tested by evaluating the transformation of Fe^{3+} to Fe^{2+}. The presence of antioxidants reduces the Fe^{3+}/ferricyanide complex to ferrous form (Fe^{2+}). The methanol and aqueous extracts showed higher reducing ability compared to the crude powder. However, the reducing ability of all samples were found to be concentration dependent when compared to ascorbic acid.

The crude powder, methanol and aqueous extract of *Digitaria exilis* grains were capable of scavenging hydrogen peroxide in a concentration dependent manner. Hydrogen peroxide occurs at very low concentration and is usually non-reactive to cells. However, it is capable of generating hydroxyl radicals which are toxic to cells by causing lipid peroxidation and DNA damage (Nandhakumar & Indumathi, 2013). Therefore, the scavenging of hydroxyl radicals is important in protecting living cells from damages. The ability of *Digitaria exilis* grains to scavenge hydrogen peroxide can be attributed to the presence of the polyphenols.

Conclusion

In conclusion, the results from this study showed that *Digitaria exilis* grains contain high amounts of total phenolic content and total flavonoid content. The presence of these polyphenols may be responsible for the antioxidant activity of the grains. It was also observed that the methanol extract showed more potent antioxidant activity compared to the aqueous extract and crude powder. The high antioxidant potential of the methanol extract may be due to differences in solvent polarity. Phytochemicals are easily extracted in more polar solvent. The present results showed the antioxidant potential of *Digitaria exilis* grains which may account for the use of the grain in traditional medicine

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