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Antioxidant potential of extracts from peels and stems of yellow-fleshed and white cassava varieties

Esther Ekeledo,[1,2,3*] Sajid Latif,[4] Adebayo Abass[5] & Joachim Müller[4]

1 Institute of Agricultural Engineering, Tropics and Subtropics Group (440e), University of Hohenheim, 70599, Stuttgart, Germany
2 International Institute for Tropical Agriculture, Ibadan, Nigeria
3 National Root Crops Research Institute, Umudike, Nigeria
4 Institute of Agricultural Engineering (440e), Faculty of Agriculture, University of Hohenheim, Stuttgart, Germany
5 International Institute of Tropical Agriculture, Regional Hub for Eastern Africa, Dar es Salaam, Tanzania

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Summary This study focused on the exploration of the potentials of extracting antioxidants from peels and stems of yellow-fleshed and white cassava varieties. The effect of particle size (0.2 and 0.5 mm) and variety on the phenolic content and antioxidant activity was assessed. The peels of the yellow-fleshed cassava variety with a particle size of 0.2 mm showed the highest phenolic content with 681.5 GAE mg 100 g^{-1} and antioxidant activity of 19% and 425 μM TE g^{-1} dry matter using DPPH and FRAP assays respectively. The stems of the white cassava with a particle size of 0.2 mm exhibited high phenolic content (442.4 GAE mg 100 g^{-1}) and antioxidant activity of 12.8% and 234 μM TE g^{-1}, better than the stem of the yellow-fleshed cassava. These results indicate that phenolic and antioxidant extractions were influenced by variety, the plant parts and particle size for the antioxidant assays.

Keywords Antioxidant activity, extracts, *Manihot esculenta*, methanolic extraction, natural antioxidants, phenols.

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important and dominant staple crops in the developing countries of tropical humid and sub-humid areas in Africa. According to de Carvalho *et al.* (2018), cassava is the fifth most important staple crop with a root production of about 285 million tons per year. In Nigeria, the average yield of cassava from over 40 cassava varieties is 10.6 t ha^{-1} (IITA, 2013). Presently, the newly bred yellow-fleshed cassava varieties known as provitamin A carotenoid (PVAC) cassava which contains a substantial quantity of β-carotene have been developed by the International Institute of Tropical Agriculture (IITA) in partnership with the National Root Crop Research Institute (NRCRI), Nigeria, with support from the HarvestPlus project (The Bulletin, 2014).

Despite being native to South America, Nigeria is its largest producer, followed by Brazil, Thailand and Indonesia, with a global cultivated area of around 2.5 Mio. ha and average productivity of 11.4 t ha^{-1} (De Carvalho *et al.*, 2011). Plants, including root tubers, are a potential source of natural antioxidants and acts as secondary metabolites. Antioxidants are present not only in the flesh but also in the peel of the cassava root (Dusuki *et al.*, 2020). According to Blagbrough *et al.* (2010), Prawat *et al.* (1995) and Reilly *et al* (2004), substantial amounts of bioactive compounds are present in cassava plants such as hydroxycoumarins, which consist of scopoletin, terpenoids and flavonoids. These compounds have several different functions such as protection against pests, the attraction of pollinators or the signalling of essential functions. They have usually been associated with plant growth and development apart from their primary biosynthesis. Natural antioxidants or phytochemical antioxidants such as phenolic, flavonoid, anthocyanin and carotenoid possess many functions that prevent deterioration of tubers as well as extend its shelf life (Ghasemzadeh *et al.*, 2012). Cassava also contains anti-nutrients such as phytate, polyphenols, tannins, oxalate, alkaloids and saponins, especially in their peels and stems, but some of these compounds can also act as anti-carcinogens and antioxidants (Anbuselvi & Balamurugan, 2014).

Cassava being a highly perishable crop requires processing immediately after harvesting leading to an abundance of residues such as peels, starch bagasse,

*Correspondent: E-mails:enekeledo@gmail.com; info440e@uni-hohenheim.de

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wastewater effluents and discarded stems, which are regarded as waste are indiscriminately dumped, thereby causing environmental pollution and health hazards (Jyothi *et al*., 2005; Cumbana *et al*., 2007). In Nigeria, about 14 million metric tonnes (Mt) of by-products, comprising peels, stumps, woody and undersized root are disposed of as waste (Okike *et al*., 2015). An estimate of about 250–300 kg peels per ton of fresh cassava root is generated (FAO, 2001).

About 144%–257% of the root weight makes up the residues of the cassava crop, which consist mainly the shoots of the plant after the harvest of the cassava roots (Viridiana *et al*., 2010). The under-utilised stems are burned, leading to environmental pollution (Zhu *et al*., 2015).

The thin pericarp and the parenchyma are the two layers that make up the cassava peel (phelloderm) and are usually removed along with some part of the pulp that adheres to the peels (Kongkiattikajorn & Sornvoraweat, 2011). Cassava peels contain crude protein between 4.1% and 6.5%, dry matter 86.5%–94.5%, organic matter 81.9%–93.9%, neutral detergent fibre (hemicellulose and cellulose) 34.4% and lignin 8.4% (Kongkiattikajorn & Sornvoraweat, 2011).

To adequately access the antioxidant capacity (TAC) in foods, several antioxidant assays that were developed may be applied. These include ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), 2,2-azino-di-3-ethylbenzthiazoline-6-sulphonate (ABTS) and total radical-trapping antioxidant parameter (TRAP) assays (Huang *et al*., 2005) but for this study, only DPPH, FRAP and TPC assays will be implemented.

To our knowledge, no studies have evaluated the antioxidant activity of the newly bred yellow-fleshed cassava variety from Nigeria and white cassava peels and stems. Thus, the exploration of the antioxidants naturally present in cassava could be economically valuable as well as of great interest for applications in functional foods through the utilisation of the abundant biomass residues (peels and stems). Therefore, the purpose of this study was to determine the presence and accessibility of antioxidant activity of the peels and stems of yellow-fleshed and white-fleshed cassava varieties and to correlate it to their phenolic content using DPPH and FRAP assays.

Materials and methods

Plant materials

Roots and stems (12 months old) of the yellow cassava variety TMS 01/1368 were collected from the IITA experimental fields in Ibadan (7.495039 N, 3.8976916 E) in the South-West of Nigeria. The average temperature is 26.5 °C. Daily temperature ranges from 23.0 to 31.7 °C with about 1,311 mm of precipitation annually. Roots of white cassava from Costa Rica were purchased from a supermarket in Stuttgart, Germany. Stems from the white cassava were obtained from a greenhouse of the University of Hohenheim, Germany.

Chemicals and reagents

1, 1-diphenyl-1-picrylhydrazyl radical (DPPH), Folin–Ciocalteu phenol reagent, Gallic acid, sodium carbonate, methanol and Trolox were purchased from Sigma-Aldrich, Taufkirchen, Germany. All the reagents were analytical and HPLC grade.

Sample preparation

The peels and stems of the two cassava varieties were used. The roots were washed after harvest and air-dried before gently scraping off the thin pericarp and parenchyma. The inner part (cortex) of the peel was peeled off in circular form to avoid losing part of the peels and mixing with the fleshy part. The peels (cortex) were dried in a cabinet dryer (Niji Lukas, Lagos, Nigeria) at 55 °C, packed in polyethene bags and stored at a room temperature of 28 °C before being shipped to the University of Hohenheim, Stuttgart (Germany), for further analysis. The matured middle parts of the stem were washed and cut into pieces of 10 cm and dried in a cabinet dryer at 55 °C. The samples were milled by a hammer mill (Niji Lukas), packed in polyethylene bags and stored at a room temperature of 28 °C before being shipped to the University for further analysis. The peel and stem of the white cassava were prepared by the same procedure; the milling of the peels and stems was done using a coffee grinder (MKM6003; Robert Bosch GmbH, Stuttgart, Germany) and a laboratory mill (Polymix PX-MFC 90 D; Kinematica AG, Luzern, Switzerland).

Sample extraction

The ground and sieved cassava peels and the stems sized 0.2 and 0.5 mm, were weighed separately into 50-mL falcon tubes and then extracted with 25 mL of 80% methanol in a thermostat-controlled ultrasonic bath (Transonic 780/H; Elma Schmidbauer GmbH, Singen, Germany) for 20 min at 25°C using the method described by Marinova *et al*. (2005). During the extraction, the tubes were held in a thermostat-controlled ultrasonic bath, and the samples were covered to prevent oxidative changes through the presence of light. The samples were centrifuged using a tabletop cold centrifuge (Z326K Hermle Labortechnik GmbH,

Wehingen, Germany) for 15 min at 4 °C at 14 000 rpm. The supernatant was then extracted carefully into another set of 50-mL falcon tubes. The extraction procedure was repeated by adding a second 25 mL of 80% methanol to the initial falcon tubes with sample residues for maximum extraction of the materials. The supernatant was collected, and the pooled extracts were stored at −23 °C for further analyses. Each sample was replicated thrice.

Antioxidant capacity

Total phenolic content assay

The total phenolic content (TPC) of the peel and stem extracts was determined using the Folin–Ciocalteu reagent (FC) and gallic acid standard by the method of Thaiponga *et al*. (2006). The FC measures the sample's reducing capacity. The ranges of sample dilution concentration values from the peel and stem extracts were chosen to fall within the limit of linearity (50 and 1000 μM) for the standard curve of gallic acid for total phenol. From the resulting diluted sample extracts, 150 μL was thoroughly mixed with 150 μL Folin–Ciocalteu reagent (0.25 N) and 2400 μL of HPLC water in a 15-mL falcon tube with a vortex mixer (Genius VG3; IKA-Werke GmbH & Co. KG, Staufen, Germany). After 3 min of incubation at room temperature (23 °C), 300 μL of 1 N sodium carbonate solution was added to the entire sample extracts and mixed well with the vortex mixer before being incubated for 2 h in the dark at room temperature (23 °C). The same procedure was repeated for the blank and the standard sample using 80% methanol and gallic acid solution, respectively, in place of the sample extracts. 150 μL of the standard was prepared using the same procedure as before but without the sample extract. It was mixed with 150 μL Folin–Ciocalteu reagent and 2400 μL of HPLC water using the vortex mixer. The dilution concentrations were 1:5 and 1:10 mg mL^{-1}, respectively. The absorbance of the standards and sample extracts was measured at 725 nm after 2 h of incubation in the dark at ambient room temperature using a UV-VIS spectrophotometer (DR-6000; Hach Lange GmbH, Düsseldorf, Germany) at a wavelength of 725 nm. Concentration was calculated in a gallic acid equivalent (GAE) mg 100 g^{-1} sample to get the standard calibration curve, and the phenolic content was expressed as gallic acid equivalents. Samples were measured in triplicates. In order to assess the best possible condition for antioxidant activity and its extraction from cassava, based on the type of variety, plant material, particle size and dilution, TPC was used as the standard since it indicates the amount and presence or absence of polyphenolic contents in plants.

Standard response. The concentration of gallic acid in the samples was calculated using the standard gallic acid calibration curve equation:

$$y = 4.8231x + 0.005 \; R^2 = 0.9981.$$

where y is absorbance and x is gallic acid concentration (mg mL^{-1}).

Total phenolic content in the samples was determined as gallic acid equivalent (GAE mg 100 g^{-1}) using the following equation;

$$TPC = \frac{c \bullet v}{m} \bullet 100$$

where TPC is the total phenolic content (GAE mg 100 g^{-1}), c is the concentration of gallic acid (mg mL^{-1}) from the calibration curve (x), v is the volume of the extract solution (mL), and m is the mass of the extract used (g).

DPPH assay

The 1, 1-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) was determined by adopting the method of Thaiponga *et al*. (2006). To prepare the stock solutions of DPPH and Trolox, 12 mg of DPPH (60 mM) was prepared in 50 mL of 80% methanol and stored at −23°C until needed. After that, 0.025 g Trolox (1 mM) was prepared in 100 mL of 80% methanol. The working solution was prepared by mixing 20 mL DPPH stock solution with 90 mL of 80% methanol to obtain an absorbance of 1.1 ± 0.02 at the wavelength of 515 nm using the UV-VIS spectrophotometer. 147 μL of each of the sample extracts, standard and blank solution (80% methanol) was added to 2800 μL of the DPPH working solution and thoroughly mixed with the vortex. Each of the samples, standard and blank mixtures, was incubated for 24 h, respectively, in the dark at room temperature (23 °C). The absorbance was read with the aid of the UV-VIS spectrophotometer at a wavelength of 515 nm. The standard calibration curve was prepared based on the range concentrations of the samples with the Trolox stock solution within the lower and upper limits of linearity of 25 and 800 μM. Samples were measured in triplicates, and the result was expressed in Trolox equivalent (TE) μM TE g^{-1} dry weight. The DPPH concentration in the samples was calculated using the Trolox calibration curve equation:

$$y = 0.0009x + 0.0219 \; R^2 = 0.9933.$$

where y is the absorbance and x is the Trolox equivalent (μM TE g^{-1}).

The following formula was then used to calculate the percentage scavenging inhibition activity of each extract of the DPPH radical;

$$\%\text{Inhibition} = \frac{A_0 - A_1}{A_0} \bullet 100$$

where A_o is the absorbance of the blank and A_1 is the absorbance of the sample extract.

FRAP assay

The ferric reducing antioxidant power (FRAP) was determined according to the method of Thaiponga *et al.* (2006). The stock solutions used were 9.69 g sodium acetate trihydrate in 50 mL of acetic acid (100%) solution with the pH adjusted to 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl.6H_2O$ solution. A fresh working solution was prepared by mixing 25 mL sodium acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl.6H_2O$ solution. The mixture was heated in a water bath to 37 °C and used immediately. About 150 μL of each of the peel extracts, stem extracts, blank (80% methanol) and the standard were mixed with 2850 μL of the FRAP working solution and incubated for 30 min in the dark. The absorbance of the coloured sample products was read at 593 nm using the UV-VIS spectrophotometer. The concentration of FRAP in the samples was calculated using Trolox standard calibration curve equation:

$$y = 0.002x + 0.0029 \quad R^2 = 0.9999$$

where y is the absorbance and x is the Trolox equivalent (μM TE g^{-1}).

The standard curve was based on the concentration ranges using Trolox as the standard. The linearity was between 25 and 800 μM, and the result was expressed in μM TE g^{-1} dry weight. The antioxidant assays were performed in triplicate for each extract.

Statistical analysis

All data were expressed as mean ± standard errors of triplicate measurements, and statistical analysis was done by SPSS for Windows (ver.16.1). One-way analysis of variance (ANOVA) and Duncan multiple range test were carried out to test any significant differences between means. An error probability value of $P \leq 0.05$ was considered to denote a statistically significant difference. The Pearson correlation coefficients of determination (R^2) were calculated to determine the relationship between the three assays and the sample extracts.

Results and discussion

Total phenolic content

The total phenolic content (TPC) from the 0.2 mm sized particles of the yellow peel (YP), yellow stem (YS), white peel (WP) and white stem (WS), and the dilution concentrations (1:5 mg mL^{-1} and 1:10 mg mL^{-1}) are shown in Fig. 1a. The TPC of the YP was significantly different ($P \leq 0.05$) from the WP for the two dilution concentrations with the YP having the highest TPC activity of 681.5 GAE mg 100 g^{-1} and 674.7 GAE mg 100 g^{-1} dry matter for 1:5 mg mL^{-1} and 1:10 mg mL^{-1} dilutions, respectively. This may be attributed to their flesh colour, the age of the plant, location and varietal differences because the yellow-fleshed cassava is a newly bred variety enriched with beta carotene, as is evident from its yellowish flesh colour. The WS and YS showed no significant differences from one another based on the dilution concentrations. In Fig. 1b, the same trend was observed for TPC from a 0.5 mm particle of both plant materials for both dilutions except for WS and YS of 1:5 mg mL^{-1} dilution. The YP extract from 1:10 mg mL^{-1} dilution was significantly higher ($P \leq 0.05$) with a higher TPC activity of 676.0 GAE mg 100 g^{-1} compared to that of the WP extract (253.4 GAE mg 100 g^{-1} dry matter) while the YS and WS were within the same TPC range with no difference. Meanwhile, observations recorded from 1:5 mg mL^{-1} dilution concentration revealed significant differences between the plant materials from both cassava varieties. Although WS showed no significant difference between the two dilutions for the stem extracts, it could be seen that the YS extract was significantly different, since the YS extract with higher dilution yielded higher TPC activity.

This TPC activity observed could be attributed to the influence caused by the dilution concentration as a slight increase in concentration corresponded to a relatively higher TPC activity. In comparing the stem extracts of both particle sizes with dilution concentrations, it can be observed that a higher TPC activity was observed in Fig. 1a than in Fig. 1b, although Fig. 1a showed no statistically significant differences unlike the result in Fig. 1b. The TPC results obtained from the YP extract were higher than the cortex layer of the cassava variety (71.79 μg GAE g^{-1}) as reported by Dusuki *et al.* (2020), and that of pomegranate peels (297.5–435 mg tannic acid equivalents g^{-1}) reported by Malviya *et al.* (2014). The variations could be due to differences in the sample materials used, their farm soil compositions, climates and the different farming environment. Dusuki *et al.* (2020) reported that the composition of different cassava roots might vary due to the factors mentioned above. The deviation observed in the assay could be attributed to the fact that the antioxidant capacity (TAC) was not independent of the quantity of sample material used during the assay and could also be due to the solvent: sample ratio, which exerted a positive effect as indicated by the high yield observed in the yellow peel and white stem, respectively. It could also be as a result of the fact that

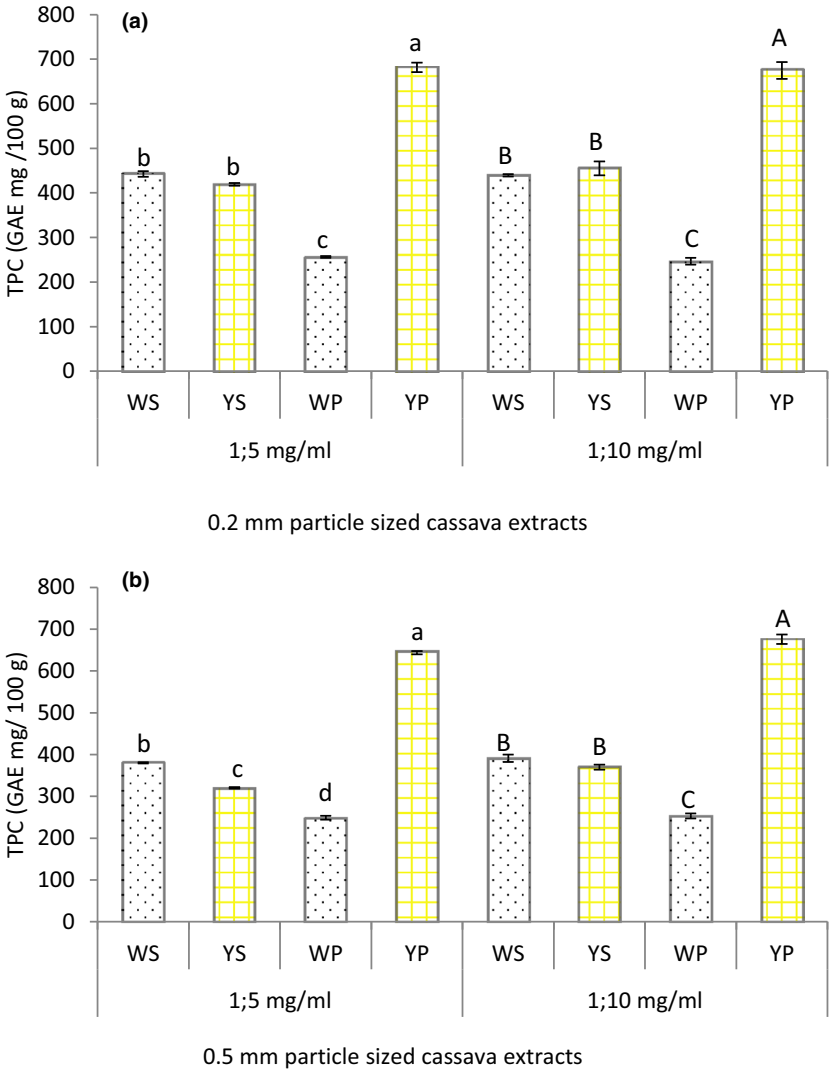

Figure 1 Total phenolic content (TPC) based on (a) 0.2-mm particle size and (b) 0.5-mm particle size of the white and yellow cassava peel and stem extracts. WP, white peels; WS, white stems; YP, yellow peels; YS, yellow stems. Different lowercase letters or uppercase letters indicate significant differences in the respective group ($P \leq 0.05$).

different plants contain several polyphenol and non-polyphenol antioxidants at various concentrations that react differently based on the source of radical assay applied. The TPC of the WP extracts from our results was relatively lower than for pomegranate but was also higher than that of the peels from purple and pigmented potato clones (3.0–12.5 GAE mg 100 g^{-1}) as reported by Ji *et al.* (2012). The TPC results of the YP extracts from our study were similar to Chinese purple yam peels (695.1 mg GAE g^{-1} dry matter) as reported by Xiao-Xuan *et al* (2015). The TPC values of the WS and YS extracts from our study were within the same range as that reported by Malviya *et al.* (2014) except for 0.2 mm particle-sized WS extracts at both dilution concentrations, and for 0.2 mm particle-sized YS extract at higher dilution concentration that had a slightly higher TPC value.

Differences in TPC have also been attributed to the cultivar/specie, environmental conditions, postharvest practices, processing and storage (Naczk & Shahidi, 2006). The presence of phenolic compounds in the peel and stem of cassava varieties due to their natural colour and varietal type (Kanatt *et al.*, 2005; Ngadze *et al.*, 2014) makes them similar to other root crops. The increase in the TPC values as indicated in the plant extracts could be due to the cell structure and chemical composition, which were broken down to allow the liberation of the bound phenolic compound

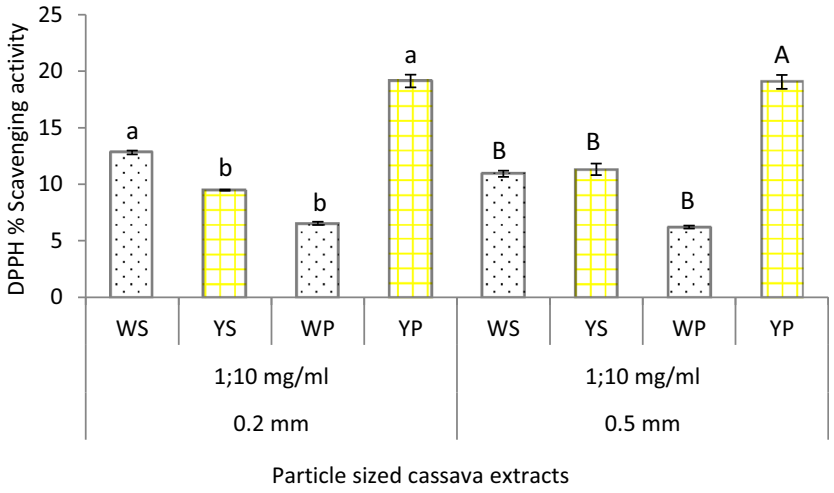

Figure 2 Percentage scavenging activity of the 0.2-mm and 0.5-mm particle sizes of the white and yellow cassava peel and stem extracts based on 1:10 mg mL^{-1} dilution concentration. WP, white peels; WS, white stems; YP, yellow peels; YS, yellow stems. Different lowercase letters or uppercase letters indicate significant differences in the respective group ($P \leq 0.05$).

from the cellular tissues (Haard & Chism, 1996). The TPC of both, the WS and YS, was in contrast to those reported by Deepanjan *et al* (2008) on the mangrove species, *Ceriops decandra* and *Avicennia alba*. The bark extract from the stem of the mangrove specie *C. decandra* had a TPC of 94.41 GAE mg 100 g^{-1} dry matter while that of the *A. alba* extract was 4.40 GAE mg 100 g^{-1}. This could probably be due to the type of plant species. The high TPC in the peels of the root crops could be an indication that the peels tend to contain more bioactive compounds as a high phenolic content is responsible for bioactivity and therefore is expected to exhibit good results in antioxidant activities.

Scavenging activity (SCA)

In Fig. 2, a significant difference ($P \leq 0.05$) was observed in the antioxidant activities among WP, and YP extracts from the 0.2 mm sized particles. The YP extract had 19% scavenging activity while the WP had an SCA of 6.5%. The opposite was true for the cassava stem extracts as the WS had 12.8% higher SCA than the YS extract (9.5%).

For the 0.5-mm particle size, the SCA 19.1% for YP extract was significantly higher than that of the WP extract of 6.2%. It was also observed that for the stem extracts of 0.5 mm particles, the YS extract had a slightly higher SCA of 11.3% than the WS extract (10.9%). The YP extract showed the best antioxidant activity (19.1%) at both particle sizes. Its efficacy was superior to the WP extract (6.5%). The SCA of YP particle sizes was not different from each other, and the same trend was observed for the WP extract as well.

However, it was observed that for the 0.5 mm particle-sized YS extract with a SCA of 11.3%, it was significantly higher than the 0.2 mm particle-sized counterpart YS that had a SCA of 9.5%. While the 0.2 mm particle-sized WS extract had a slightly higher SCA of 12.8% than the 0.5 mm particle-sized WS extract that had a SCA of 10.9%. The differences observed in both varieties could be an indication of varietal differences enhanced due to particle size effect as it can be seen that the yellow cassava stem, unlike the white cassava stem, required higher particle size for the maximum extraction. The DPPH scavenging activity (SCA) of the extracts had low values compared to the results reported by Chidambara *et al* (2002), the YP seemed to have a higher scavenging activity than the YS, WS and WP, although much lower than that of the pomegranate peel (81%). This could be attributed to their different genre and phytochemical composition. According to Ji *et al.* (2012), the peel of the potato clones exhibited the most potent antioxidant activity while Dilworth *et al.* (2012) reported that water yam and dasheen had the highest percentage of DPPH inhibition activity with 95.83% and 93.41%, respectively. The high values for the antioxidant activity may be attributed to high levels of phenols and flavonoids coupled with other compounds such as phenylpropanoids and anthocyanin (Spina *et al*., 2008). The results obtained from the WS were in conformity with the notion that a reduction in particle size leads to increase in extraction yield as a relatively slight increase was observed between the two-particle sizes while for the YS, the effect was in direct dissimilarity as reducing the particle size led to a decrease in the SCA even though no statistical difference was shown.

Ferric reducing antioxidant power (FRAP)

Figure 3 shows the FRAP of the peel and stem extracts of both the white and yellow varieties. Irrespective of particle size, the FRAP of YP extract was

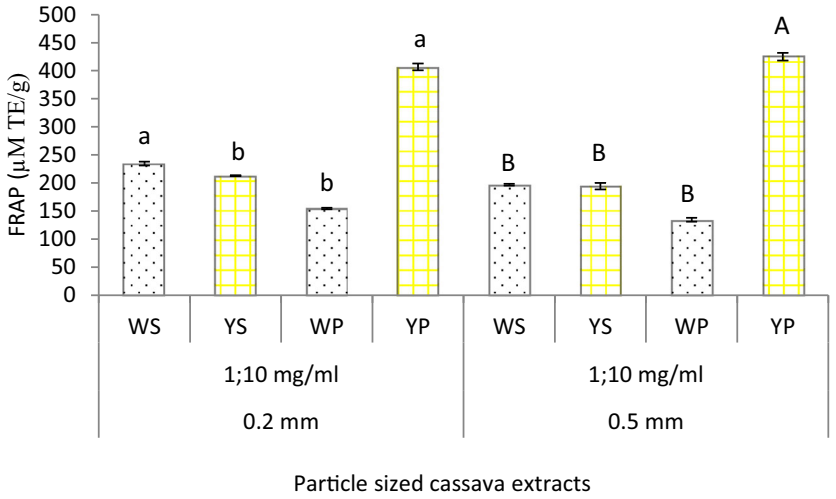

Figure 3 Ferric reducing antioxidant power of the 0.2-mm and 0.5-mm particle sizes of the white and yellow cassava peel and stem extracts based on 1:10 mg mL^{-1} dilution concentration. WP, white peels; WS, white stems; YP, yellow peels; YS, yellow stems. Different lowercase letters or uppercase letters indicate significant differences in the respective group ($P \leq 0.05$).

significantly higher ($P \leq 0.05$) than the FRAP of all the other sample extracts except WS extract at 0.2 mm. All the remaining extracts have comparable FRAP values. In the FRAP results obtained (Fig. 3), it was observed that the YP of both particle sizes was significantly higher than the WP, YS and WS. Based on the intraparticle diffusion theory, the YS and WS met the criteria as they had more ferric reducing power when the particle sizes were reduced to 0.2 mm. The differences observed could be that the antioxidant activities (AA) in plant materials are dependent on the solvent: sample ratio utilised during the assay. This was also evident in the reports obtained from Fukumoto & Mazza (2000), Cao *et al.* (1997), Saint-Cricq de Gaulejac *et al* (1999) and Van den Berg *et al.* (1999). They reported that the TEAC values are concentration-dependent in accordance with the linearity of the antioxidant activity, which can only increase by concentration up to a certain limit, after which more concentration increments above the linearity range will lead to a lower or no yield. The origin of the sample materials, their compositions and probably the solvent used in the extraction could also be a contributing factor.

Dilution concentration (DC) and particle size (PS) effects

According to Stalikas (2007), the chemical nature of the plants' phytochemicals, the extraction method applied, the sample particle sizes, the solvent used and the presence of interfering substances all influence the extraction efficiency. Figure 1a,b showed the effect of PS against DC. The TPC of the WP extract for 0.2 mm PS at a low concentration was slightly higher than the TPC for 0.5 mm PS. A different trend was observed for the same PS at a higher DC except for WP and YP extracts that had similar TPC for both (individual) particle sizes, respectively. 0.2 mm PS samples from the result exhibited good antioxidant activity, and this could be related to the fact that the high antioxidant activity is positively correlated with the TPC activity. To determine the best condition for antioxidant activity and extraction in cassava based on variety type, plant material, particle size and dilution, TPC was used as an indicator since it indicates the amount and presence or absence of polyphenol contents in plants. The results from this study showed that YP extracts gave the highest TPC followed by the WS. The results obtained from the YP and WP extracts with low dilution concentration based on the particle size, and dilution concentration can be seen to be closely related to the intraparticle diffusion theory. It was observed that the TPC of these extracts increased at a low dilution concentration when the particle sizes were reduced from 0.5 mm to 0.2 mm. It is also important to note that a high phenolic content and antioxidant activity during extraction may be attributed to other compounds such as proteins and carbohydrates that are highly soluble in methanol (Zielinski & Kozøowska, 2000). Other factors that may contribute to high phenolic content in plants might include cultivation practices and genotypic differences (Weston & Barth, 1997; Asian Vegetable Research & Development Center, 2003; Rekika *et al.*, 2005). Bok *et al* (2006) reported that different species are known to have different levels of antioxidant compounds. The dilution concentration could also be a contributing factor as observed in the TPC result as the changes occurred due to its concentration-dependent relationship and from the physical and biochemical interactions between the antioxidants present in the sample extracts (Freeman *et al.*, 2010).

To determine the linearity range, different dilution concentration values of the sample extracts and

Table 1 Pearson correlation coefficient of total phenolic content, scavenging activity and ferric reducing antioxidant power assays of the cassava peel and stem extracts

	DPPH	FRAP
TPC	0.908*	0.920*
DPPH	–	0.972*

DPPH, scavenging activity; FRAP, ferric reducing antioxidant power; TPC, total phenolic content.
*Correlation is significant at 0.01 level (2 tailed).

standard solutions were carefully selected prior to the TP assay using gallic acid as a standard. The deviation observed in the assay could be attributed to the fact that the antioxidant capacity (TAC) values depended on the quantity of the sample: solvent ratio used which exerted a positive effect as indicated by the high antioxidant yields observed in the YP, YS and WS extracts, respectively. It could also be a result of the fact that different plants contain polyphenols and antioxidants at various concentrations.

Pearson correlation coefficient of TPC, DPPH and FRAP assays

Correlation analysis on the antioxidant and the total phenolic contents (TPC) of yellow-fleshed and white cassava peel and stem extracts (Table 1) was performed. The Pearson correlation coefficient of TPC, DPPH and FRAP assays revealed a statistically significant linear relationship ($P \leq 0.01$). The extracts of white and yellow cassava peels and stems were highly correlated at $R^2 = 0.908$; 0.920; and 0.972 for TPC-DPPH; TPC-FRAP; and DPPH-FRAP, respectively. TPC was positively and strongly correlated with DPPP and FRAP antioxidant activity, and therefore, it may be concluded that the antioxidant activity of the peel and stem extracts is related to the active component. Different correlation analysis between TPC and antioxidant activities (DPPH and FRAP) can be found in the literature (Mahattanatawee *et al*., 2006). The positive and strong correlation observed in this study was in agreement with the results of many research studies such as Deepanjan *et al* (2008) and Katalinic *et al* (2006) that reported a significant correlation between TPC and ferric reducing antioxidant power.

Conclusion

Data obtained in this study highlighted significant variations in the antioxidant activities between the peels and stems of the two cassava varieties (yellow- and white-fleshed) and also showing a strong correlation between the assays. The smaller particle size (0.2 mm) gave, the higher TPC, and antioxidant activity across the three assays in the following order YP > WS > YS > WP extracts. YP and WS extracts exhibited the most significant antioxidant activity at a low dilution concentration, while the 0.2 mm particle-sized YS extract exhibited a higher antioxidant activity. For the other antioxidant assays, a variation of the particle sizes with less dilution concentration occurred as it highly depends on the linearity limits of the standard curve for the assay used. The peel from the yellow-fleshed cassava variety may be considered as a good source of natural antioxidants, and its availability as a by-product may be an added advantage. It can be promoted for the application as a food additive in functional foods and fat-based cosmetics. Their utilisation can serve in reducing the waste disposal problem and hence protect the environment.

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Conflict of interest

The authors declare that this manuscript is original and has not been published nor is it under consideration for publication; elsewhere, we also declare that there are no conflicts of interest with respect to the work in this manuscript.

Author contribution

Esther Ekeledo: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Writing-original draft (lead). **Sajid Latif:** Project administration (supporting); Supervision (supporting); Validation (supporting); Writing-review & editing (supporting). **Adebayo Abass:** Funding acquisition (lead);

Supervision (supporting). **Joachim Muller:** Data curation (equal); Resources (lead); Supervision (lead); Validation (supporting); Writing-review & editing (lead).

Ethical approval

Ethics approval was not required for this research.

Peer Review

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Research data are not shared.

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