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Determination of the Antioxidant Potentials of Two Different Varieties of Banana Peels in Two Different Solvents

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Abstract

Fruit wastes which are perishable pose a big problem to pollution monitoring agencies; however these problems can be reduced by utilizing the high value compounds present in the food waste. Banana peels contain a reasonable number of antioxidant compounds such as gallocatechin and dopamine. This paper investigates the antioxidant potential of two different varieties of Banana peels (Dwarf cavandish and Musa omini). The peels were extracted with two different solvents (Methanol and Ethanol) using the soxhlet extractor. The results show that ethanolic extracts have higher phenolics and flavonoid contents (336.83 - 383.83 mgGAE/100g, 242.83 - 252.82 mgRutin/100g) compared to the methanolic extracts of the same banana varieties (299.42 - 344 mgGAE/100g, 240.77 - 241.23 mgRutin/100g). However methanolic extracts exhibit higher DPPH Antioxidants Activity (30.82% - 51.66%) compared to ethanolic extracts (25.44% - 30.27%). This implies that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the DPPH Radicals. It was also observed that at any concentration between 0.5 - 2.5 mg, ethanol extracts of both dwarf cavandish and musa omini had higher reducing power than the other two varieties.

Keywords

Antioxidants, Banana Peels, Phenolics, Extracts, Fruit Waste

1. Introduction

Fruit wastes are highly perishable and seasonal and they create problems to the

processing industries and pollution monitoring agencies. These problems can be reduced by utilizing its high value compounds, including the dietary fibre fraction that has a great potential in the preparation of functional foods [1]. The peels of a variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity [2]. Banana peel, an underutilized source of phenolic compounds is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease [3]. The peel of the fruit contains various antioxidant compounds such as gallocatechin and dopamine [1]. Recent trends focus on the isolation, characterization and utilization of natural antioxidants, especially growing interest in polyphenols as potential disease preventing agents. As these compounds are predominantly found in most of fruit tissues, it would be worthwhile investigating the nature of polyphenols that are present in banana peel, a potential source of antioxidant and antimicrobial activities. Antioxidants are important additive in gasoline; these antioxidants prevent the formation of gums that interfere with the operation of internal combustion engines [4].

An antioxidant is any substance which when present at low concentration compared to that of oxidizable substrates, significantly delays or inhibits oxidation of the substrate [5]. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent; oxidation reaction can produce free radicals. In turn, these free radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell [4]. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions; they do this by oxidizing themselves, so antioxidants are reducing agents such as thiols, ascorbic acids or polyphenols [6]. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Numerous methods have been proposed for measuring the antioxidant activities of food wastes; however the essential feature of any method includes the presence of a substrate, an oxidation initiator and an appropriate measurement of the end point. A simple and inexpensive method that can be used to analyze and measure the antioxidant capacity of food wastes involves the use of free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The molecule of DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would have been the case with most other free radicals [7]. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol or methanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to a reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present) [8].

This paper elucidates the antioxidant activity of banana peel extracts, and it also analyzes the relationship between the phytochemical content and free radical scavenging activity of banana peels using the DPPH free scavenging activity and reducing power assay.

2. Materials and Methods

2.1. Feedstock Preparation

Two varieties of banana dwarf cavanish and paranta (musa omini) were purchased from the "Uselu" market in Benin-City, Edo state, Nigeria. The peels were washed with distilled water and oven dried separately at 50˚C and powdered using a lab grinder and stored in air tight jar at 4˚C until use.

2.2. Preparation of Ethanolic Extracts

32.24 g of powdered peel was packed in thimble flask and 550 ml of ethanol (70%) was added in 1 liter round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles of extraction. After that, the extract was filtered and filtrate was concentrated up to 50 ml using water bath. From the concentrated 10 ml of extract was taken in evaporating dish (Borosilicate glass) which is previously weighed. The total weigh of evaporating dish containing 10 ml extract was recorded and the extract was evaporated till thick liquor was obtained. Thereafter, the calculated difference in weight was noted at every 10 min until the constant weigh was obtained. The residue at the constant weight is used as dry extractives, and stored until further use.

2.3. Preparation of Methanolic Extracts

70.98 g of powdered peel was packed in thimble flask and 550 ml of methanol (70%) was added in 1 liter round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles of extraction. After that, the extract was filtered and the filtrate was concentrated up to 50 ml using a waterbath. From the concentrated sample, 10 ml of extract was taken in evaporating dish (Borosilicate glass) which is previously weighed. The total weigh of the evaporating dish containing 10 ml extract was recorded and the extract was evaporated till thick liquor was obtained. Subsequently, the difference in weight was noted at every 10 min until the constant weigh was obtained. The residue at the constant weight is used as dry extractives, and stored until further use.

2.4. Preparation of Gallic Acid Standard Curve

Gallic acid stock solution was prepared by dissolving 0.2 g of dry gallic acid in 10 ml of methanol and diluted to a volume of 100 ml with distilled water. For the sodium carbonate solution, 20 g of anhydrous sodium carbonate was dissolved in 80 ml of distilled water and brought to boil. In order to prepare the calibration curve, 0, 1, 2, 3, 4, and 5 ml of the above stock solution in 100 ml volumetric flasks were diluted to volume with distilled water. These solutions will have concentrations of 0, 20, 40, 60, 80 and 100 mg/l gallic acid. For each calibration solution, pipette 0.5 ml, to each solution, 2 ml of distilled water and 0.5 ml of Follin-Ciocalteu reagents were added and mixed well, for 8 minutes before adding 0.5 ml of sodium carbonate solution for thirty minutes at 40˚C. Absorbance of each solution is measured using a spectrometer at 765 nm and a standard curve was obtained by plotting absorbance against the gallic acid concentration.

From the curve obtained, the total phenolic contents of banana peel extracts were determined by comparing its absorbance with standard values.

2.5. Preparation of Quercetin Standard Curve

Quercetin stock solution was prepared by dissolving 0.2 g of dry quercetin in 10 ml of methanol and diluted to a volume of 100 ml with distilled water. To prepare the calibration curve, 0, 1, 2, 3, 4, and 5 ml of the above stock solution in 100 ml volumetric flasks were diluted to volume with distilled water. These solutions will have concentrations of 0, 20, 40, 60, 80 and 100 mg/l quercetin. For each calibration, pipette different amount of sample in 3.0 ml of distilled water to which 0.3 ml of 5% sodium nitrite was added and properly mixed. After 5 minutes at room temperature, 0.6 ml of 10% Aluminium chloride was added; 2 ml of 1 M sodium hydroxide was added after 6 minutes and absorbance was read at 510 nm. A standard curve was obtained by plotting absorbance against the quercetin concentration. From the curve obtained, the total flavonoid contents of banana peel extracts were determined by comparing its absorbance with standard.

2.6. Analysis of the Total Phenolics Contents (TPC)

Samples were analyzed for total polyphenol content according to the Folin-Ciocalteu method [9]. To 0.5 ml aliquot of the extract solution, 0.2 ml of Folin-Ciocalteau reagent, and a saturated solution of Na_2CO_3 (0.5 ml) was added. This was increased to 10 ml with distilled water and incubated at 27˚C for 30 min. Optical density was measured at 765 nm using a spectrophotometer. The concentration was calculated using gallic acid as a standard and the results were expressed as gallic acid equivalents/100g of sample.

2.7. Analysis of the Total Flavonoid Contents (TFC)

TFC of the extracts were determined according to the colorimetric assay following the procedure of Sultana *et al.* (2008) [10] with some modifications. 1 milliliter of aqueous extract containing 0.01 g/ml of dry matter was placed in a 10 ml volumetric flask, and then 5 ml of distilled water was added. At zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.6 ml of (10% w/v) $AlCl_3$ was added. After another 5 min, 2 ml of 1 M solution of NaOH was added. After that, the volume was made up to 10 ml with distilled water. The mixture was shaken vigorously and the absorbance of the pink color of mixture was read at 510 nm using a UV-visible spectrophotometer. A calibration curve was prepared using a standard solution of quercetin within the range 10 - 100 ppm (R2 = 0.9962) and the results were expressed as mg quercetin equivalents/100g of dry matter. All samples were analyzed in triplicates and results averaged.

2.8. Reducing Power

In this assay, Fe^{3+}/ferricyanide complex is reduced to the ferrous form by antioxidants. The Fe^{2+} formed is monitored by measuring the formation of Perl's Prussian blue at 700

nm [11]. Different amounts of sample in 1.0 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50˚C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) were mixed and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.9. Free Radical Scavenging Activity Using DPPH

The antioxidant capacity of the banana peel extracts was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was assessed by using procedure reported by Sultana *et al.* (2008) [10] with slight modifications. 5.0 ml of a freshly prepared solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanolic solution at concentration 0.025 g/l was added to 1.0 ml of extract containing 25 µg/ml of dry matter in methanol. The mixture was shaken and kept in the dark and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 515 nm, against a blank of methanol without DPPH, using a UV-visible spectrophotometer. Results were expressed as percentage of inhibition of the DPPH radical which was calculated according to the following equation: Radical scavenging activity (%) = (Control OD − Sample OD/Control OD) × 100).

3. Results and Discussion

3.1. Physical Characteristics of the Peels

The physical characteristics of the peel are given in Table 1. Fresh musa omini banana peels were yellow in colour while dwarf cavandish were green in colour; however, significant darkening was observed on drying both peels. The extract yield of powdered peel obtained was more in musa omini (8.97% - 15.41%). Similarly ethanol extracts produces more yield than methanol extracts in dwarf cavandish peels while methanol extracts produces more yield in musa omini peels. Variations in yields of extracts might be attributed to the availability of different extractable components, defined by the chemical composition, nature of the soil and agro-climatic conditions [12].

3.2. Analysis of the Total Phenolic Contents (TPC)

Table 2 shows the TPC of two different varieties of banana peel extracts using Gallic

Table 1. Physical characteristics of the peel.

Sample	Colour		Weight of sample (g)	Yield %	
	Fresh	Dry		Methanol extracts	Ethanol extracts
Musa omini (paranta)	Yellow	Brown	70.98	15.41	8.97
Dwarf cavandish	Green	Brown	70.98	3.10	3.32

Table 2. Total phenolic contents of two different varieties of banana peels in different extracts.

Solvents	Banana varieties	
	Total phenolic content (mg gallic acid/100g sample)	
	Dwarf cavandish	Musa omini (paranta)
Methanol	344.29 ± 30.86	299.42 ± 21.21
Ethanol	383.33 ± 29.46	336.83 ± 17.40

Each value is expressed as the mean ± standard error (n = 3).

Table 3. Total flavonoid contents of two different varieties of banana peels in different extracts.

Solvents	Banana varieties	
	Total flavonoid content (mg quercetin/100g sample)	
	Dwarf cavandish	Musa omini (paranta)
Methanol	241.23 ± 30.86	240.77 ± 21.21
Ethanol	252.82 ± 29.46	242.83 ± 17.40

Each value is expressed as the mean ± standard error (n = 3).

Acid standard curve with R^2 = 0.993. Phenolics contents for the banana peel samples analyzed in this study ranges from 299.42 to 383.33 GAE/100g samples. Phenolics contents in ethanol extracts varied from 336.83 to 383.33 mg GAE/100g samples. Compared to ethanol extracts, methanol extracts showed less phenolics contents which ranged from 299.42 - 344.29 mgGAE/100g samples.

3.3. Analysis of the Total Flavonoid Contents (TFC)

The TFC of the peels are given in Table 3. Flavonoid contents of banana peel samples analyzed in this sample ranges from 240.77 mg Rutin/100g sample to 252.82 mg Rutin/100g samples. TFC in ethanol extracts varied from 242.83 to 252.82 mg Rutin/100g samples, compared to ethanol extracts, methanol extracts showed less flavonoid contents which ranged from 241.23 to 240.77 mg Rutin/100g samples.

3.4. Reducing Power Assay

Figure 1 shows the relationship between the reducing power of two different varieties of banana peel extracts. It was observed that at any concentration between 0.5 - 2.5 mg, ethanol extracts of both dwarf cavandish and musa omini had higher reducing power than the other two varieties. Similarly, from Table 1, it can be observed that the ethanolic extracts of both banana peel varieties had high phenolic contents than their corresponding methanolic extracts, hence there is a close relationship between the amount of total phenolic content and the reducing power. Also the reducing power of the extracts increases slightly with an increase in concentration as shown in Figure 1. Hence, it is necessary to determine the reducing power of phenolics contents to elucidate the relationship between their antioxidant effects and their reducing power [13].

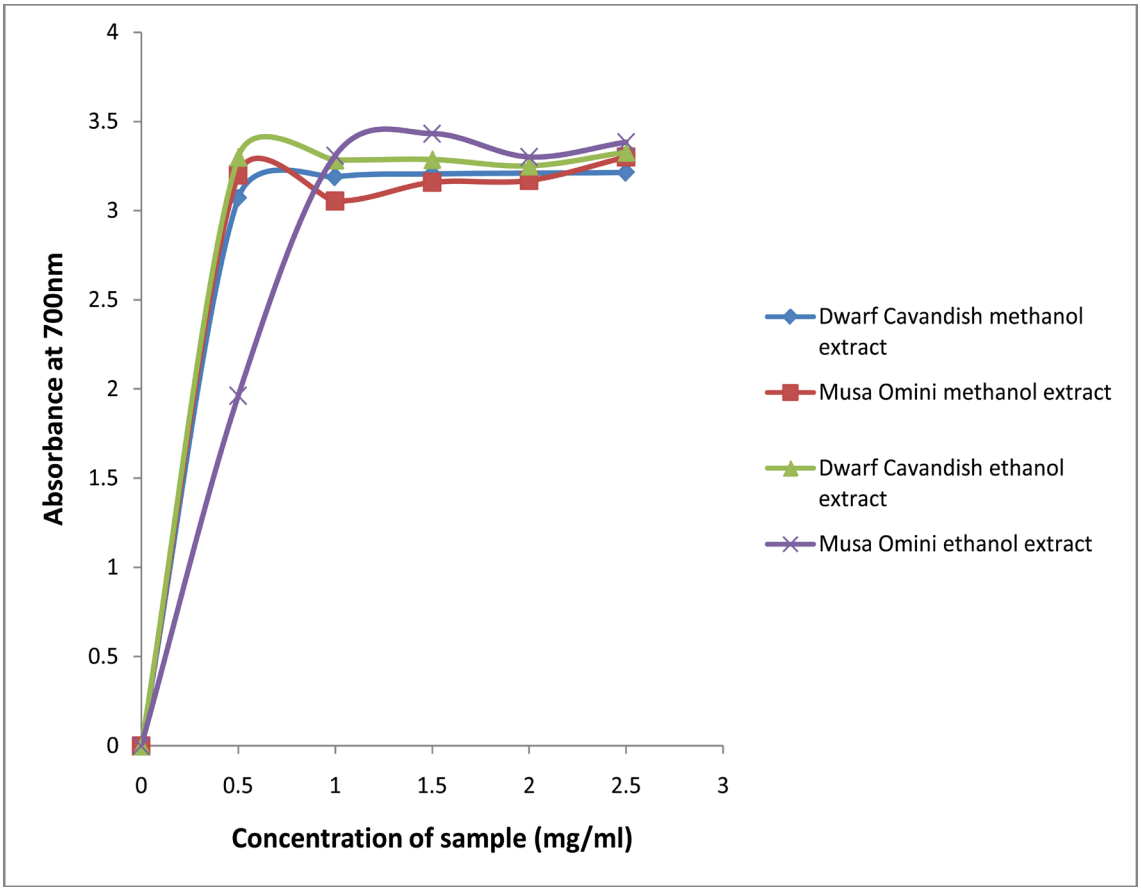

Figure 1. Reducing power of two varieties of banana peels in different extracts.

3.5. Free Radical Scavenging Activity

DPPH Free Radical Scavenging Activity is plotted as a function of a sample concentration in **Figure 2**. The inhibition of DPPH Radical of the banana peel ranged from 2.2% to 51.66%. Methanol extracts of musa omini showed higher activity (51.66%) compared to ethanol extracts (30.27%). A similar trend was observed in the case of dwarf cavandish peel where methanol extracts had an activity of (30.82%) compared to dwarf cavandish extracts (25.44%). This is consistent with those reported by Alothmant *et al.*, (2009) [14], Choo and Azis (2010) [15]. Among the two varieties, musa omini peel exhibited higher antioxidant activity at lesser concentration. As reported in literature, the strong antioxidative properties of banana extracts could be due to different antioxidant components present [1]. DPPH inhibition should follow a similar order of the total phenolic and flavonoid contents, *i.e.* as the concentration of the phenolics compounds or the degree of hydroxylation of the phenolics compounds increases, the DPPH Radical Scavenging Activity also increases [14]. This is however not the case in this study, even though musa omini and dwarf cavandish were ranked quite low in terms of TFC and TPC; their antioxidant activities were higher compared to that of the ethanolic extracts of both varieties of banana peels. This implies that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the DPPH Radicals.

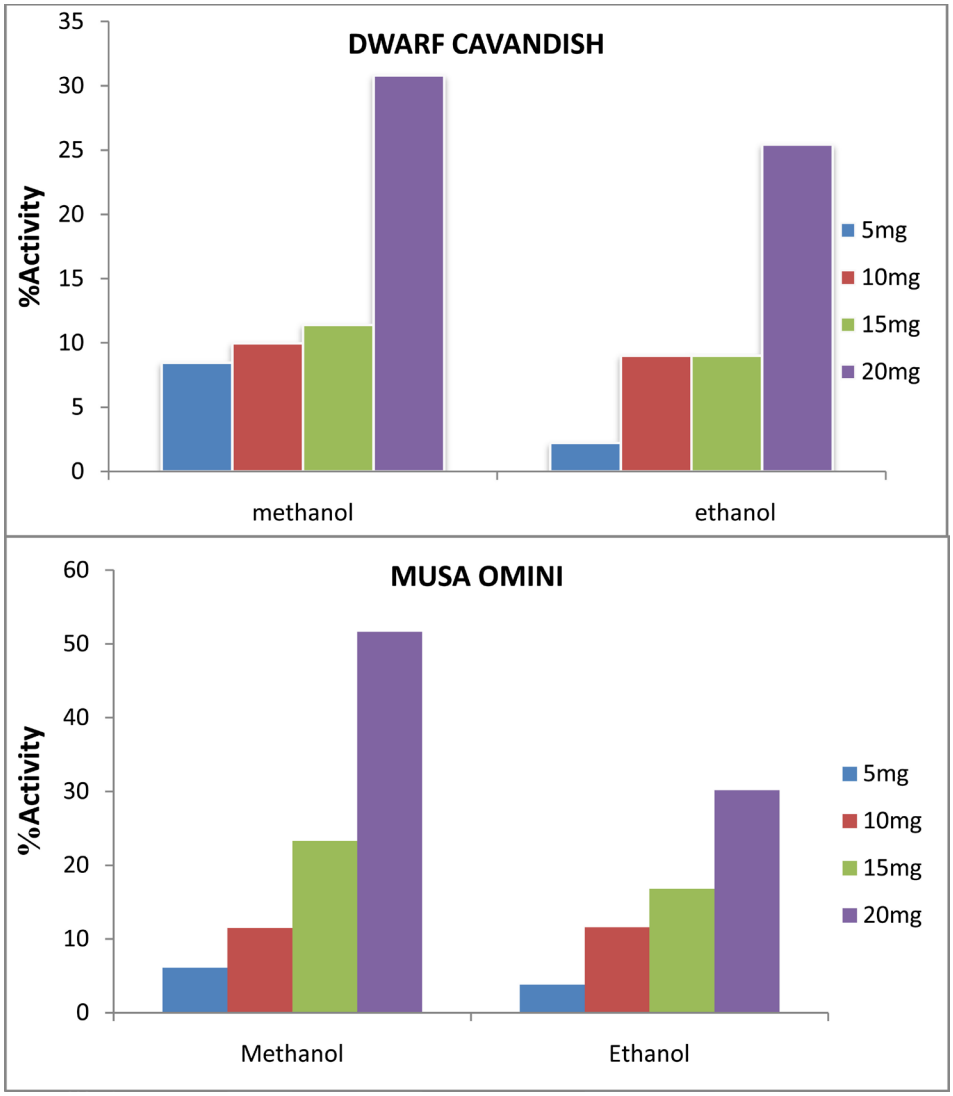

Figure 2. Free radical scavenging activity in different banana peel extracts.

Compounds such as ascorbic acids, *α*-carotene and different xanthophylls have been detected in banana and may have contributed to the antioxidant activity of the extracts [16].

4. Conclusion

This study shows that the total phenolic and flavonoid contents were higher in ethanolic extracts compared to methanolic extracts. The ethanolic extracts of Dwarf cavandish and Musa omini banana peels also have higher reducing power compared to methanol extracts of the same varieties; however methanolic extracts exhibit higher antioxidants activity compared to ethanolic extracts. This implies that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the DPPH radicals. It can thus be concluded that solvents play a vital role in the extraction of plant constituents.

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