Quantification of Total Phenolics, Flavonoids and Antioxidant Activities of Three Selected Tropical Fruits Grown In Nigeria

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Abstract
Antioxidant activity, total phenolic and flavonoid contents of tissues of three tropical fruits (pineapple, soursop and African bush mango) grown in Nigeria were determined. Samples of pineapple peels and pulp, soursop and African bush mango pulp and seeds were freeze-dried and methanolic extracts prepared using ultrasound assisted extraction method. Qualitative phytochemical screening for antioxidants was carried out in addition to quantification of total phenolics and flavonoids using Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively. Antioxidant activity of the fruit tissue extracts was determined using FRAP and DPPH assays. Data obtained were analysed using ANOVA at $p<0.05$. Qualitative screening showed the presence of phenolics, flavonoids, saponins, tannins, alkaloids, glycosides and carbohydrates in all the samples. Total phenolic content ranged from 61.5±0.12 mg GAE/g dw in African bush mango pulp to 550.7±0.33 mg GAE/g dw in Soursop pulp. Soursop pulp exhibited the highest flavonoids content (224.7±3.48 mg QE/g dw) while African bush mango pulp (43.1±2.91 mg QE/g dw) had the lowest. DPPH assay showed that Soursop pulp had the highest free radical inhibition (84.3%±0.26), while the lowest inhibition (52.1%±1.75) was obtained in African bush mango pulp. FRAP values ranged from 7.19±1.83 µM in African bush mango pulp to 28.59±0.18 µM in pineapple peels. The peels, seeds, and pulps of the selected tropical fruits contain substantial antioxidant activity and several bioactive substances that could serve as functional foods with the potential to be utilised in nutraceutical industries that could thereby have positive influence on the health of consumers.

Keywords: Phytochemical, Pineapple, African bush mango, Soursop, Tropical fruits

Quantification Des Phénols Totaux, Des Flavonoïdes et Des Activités Antioxydantes de Trois Fruits Tropicaux Sélectionnés Cultivés au Nigeria

Résumé
L'activité antioxydante, le contenu phénolique et flavonoïde total des tissus de trois fruits tropicaux (ananas, soursop et mangue de brousse africaine) cultivés au Nigeria ont été déterminés. Des échantillons de pelures et de pulpe d'ananas, de soursop et de pulpe et de graines de mangue de brousse d'Afrique ont été lyophilisés et des extraits méthanoliques ont été préparés à l'aide d'une méthode d'extraction assistée par ultrasons. Le dépistage phytochimique qualitatif

des antioxydants a été effectué en plus de la quantification des phénols totaux et des flavonoïdes au moyen de méthodes colorimétriques Folin-Ciocalteu et chlorure d'aluminium, respectivement. L'activité antioxydante des extraits de tissus de fruits a été déterminée à l'aide de tests FRAP et DPPH. Les données obtenues ont été analysées à l'aide d'ANOVA à la p0,05. Un examen qualitatif a révélé la présence de phénoliques, de flavonoïdes, de saponines, de tanins, d'alcaloïdes, de glycosides et de glucides dans tous les échantillons. La teneur totale en phénols variait de 61,5 0,12 mg GAE/g poids sec dans la pulpe de manguier d'Afrique à 550,7 0,33 mg GAE/g poids sec dans la pulpe de Soursop. La pulpe de soursop présentait la teneur en flavonoïdes la plus élevée (224,7 3,48 mg QE/g poids sec), tandis que la pulpe de mangue d'Afrique (43,1 2,91 mg QE/g poids sec) présentait la plus faible teneur. L'essai de DPPH a montré que la pulpe de Soursop avait la plus forte inhibition des radicaux libres (84,3% 0,26), tandis que la plus faible inhibition (52,1% 1,75) a été obtenue dans la pulpe de mangue de brousse africaine. Les valeurs de FRAP variaient de 7,19 1,83 µM dans la pulpe de mangue d'Afrique à 28,59 0,18 µM dans les pelures d'ananas. Les pelures, les graines et les pulpes des fruits tropicaux sélectionnés contiennent une activité antioxydante importante et plusieurs substances bioactives qui pourraient servir d'aliments fonctionnels pouvant être utilisés dans les industries nutraceutiques et avoir ainsi une influence positive sur santé des consommateurs.

Mots-clés : Phytochimique, Ananas, Mangue d'Afrique, Soursop, Fruits tropicaux

Introduction

Fruits are consumed raw or processed into either juice or smoothie, and are always available all year round in Nigeria. However, the type of fruit available at any point in time during the year is season dependent. Fruits are an excellent and natural source of nutrients, antioxidants and non-nutritive bioactive compounds in human diets (Wolfe and Ijeoma, 2010, Ghasemzadeh *et al*., 2010). Several epidemiologic studies have indicated that increased consumption of fruits is linked with reduced risk of chronic non-communicable diseases (Cohen et al., 2000; Feskanich *et al.,* 2000; Bazzano *et al*., 2002; Liu, 2003; Lunet *et al.,* 2005). The ability of fruits to reduce risks of non-communicable diseases has been attributed constituent antioxidants and non-nutritive bioactive substances such as polyphenols (Liu, 2003; Cartea *et al.,* 2011). The non-nutritive bioactive substances were reported to be the most active antioxidants (Liu, 2003). They have the capacity to fight against free radicals and other reactive oxygen species, thereby reducing oxidative stress that have been implicated in the etiology of many chronic non-communicable diseases such as type-2 diabetes and cardiovascular diseases (Cui *et al*., 2004; Veridina, 2013). In the light of the rising prevalence of oxidative stress induced diseases such as cancer and diabetes in Nigeria (Sowunmi et al., 2018; Bray et al., 2018), it is crucial to scrutinise the phytochemical profile and antioxidant properties of fruits grown in Nigeria for their potential health benefits. Data on phytochemical profile and antioxidant properties of many tropical fruits grown in Nigeria are scanty.

African bush mango (*Irvingia gabonensis* O'rorke), also called *wild mango,* is a species of *African* trees. It is indigenous to most tropical countries, especially southern Nigeria, southern Cameroon, Côte d'Ivoire, Ghana, Togo, and Benin Republic. Apart from the edible pulp of African bush mango, the seeds are also valuable for its use in the preparation of soup delicacies known as '*Ogbono'* in Nigeria. Soursop (*Annona muricata* L.), also known as graviola, is native to the tropical region. It is a dark green, spiny

aggregate fruits made up of berries fused together with associated flower parts. The pulp of the fruit contains white fibrous juicy segments and smooth hard black seeds that are oval in shape (FAO, 2011). Pineapple (*Ananas comosus* (L.) Merr) is an edible fruit that is native to the tropical region belonging to the Bromeliaceae family (FAO, 2011).

In many developing countries such as Nigeria, fruit peels and seeds are often discarded and treated as wastes. These consequently add to the volume of generated municipal solid waste and contribute significantly to the current severe menace of environmental pollution. Hence, having a value added use for fruit peels and seeds would be a step in the right direction. If these fruit wastes are found to contain significant amount of bioactive substances and antioxidant properties, the bioactive constituents, especially the phenolic compounds, can be recovered and used as natural antioxidant additives in foods for human consumption. Therefore, this study was aimed at evaluating the phytochemical composition and antioxidant activity of the seeds and pulps of selected tropical fruits - African bush mango, soursop and pineapple fruits, grown in Nigeria.

Materials and methods

Sample collection and preparation

Matured African bush mango (*Irvingia gabonensis*), soursop (*Annona muricata)* and pineapple *(Ananas comosus)* fruits were purchased from two major fruit markets (Oje and Bodija fruit markets) in Ibadan, south-west Nigeria. The fruits were properly identified in the Herbarium, Department of Botany, University of Ibadan, Nigeria. The fruits were sorted to remove the spoilt and physically damaged samples and later rinsed with distilled water to remove dirt and adhering soil particles. The peels, seeds and pulp of the fruits were manually separated using a stainless knife. The peels of African bush mango and soursop were discarded, while the peels of pineapple were further processed. The seeds (after opening of the pericarp) and pulp of African bush mango and that of soursop fruits were sliced into small pieces and put in airtight plastic containers with proper tags. The peels and pulp of pineapple were also reduced to small pieces and kept in airtight containers separately. The peels, pulps and seeds were separately freeze-dried in a lyophiliser (model YK – 118, True Ten Industrial Co. Ltd, Taiwan) for an average of 25 days until the desired dryness was achieved. The freeze-dried samples were later pulverised into powder using laboratory grinder (Thomas Scientific mini-miller; Model 3483-L70) for analysis.

Preparation of fruit extracts for analysis

Extraction procedure was carried out using ultrasound assisted extraction method as described by Altemimi *et al.* (2016) with little modifications. To 1 g of each sample measured into a 50ml centrifuge tube, 10 ml of 72% (v/v) aqueous methanol was added. The mixture was vortexed for 1 min and later put in a sonicator at ultrasonic frequency of 40 KHz at 40°C for 10 min. The resultant mixture was then centrifuged at 3000 x g for 20 min at 4ºC. Supernatants from the mixture were decanted and filtered through Whatman filter paper (No 42) and repeated in triplicates for all samples. All chemicals used were of analytical grade.

Qualitative screening for phytochemicals

Methanolic aqueous fruit extracts were assessed for the presence of different phytochemicals using standard methods as described by Debiyi and Sofowora (1978), and Trease and Evans (2002).

Phenolics

To 4 mL of the crude fruit extract, 2 mL of 2% $FeCl_3$ was added. Formation of a black or blue-green colour indicated the presence of

phenolic compounds.

Flavonoids
Concentrated HCl (5 mL) added to pieces of magnesium ribbon was mixed with 2 mL of the crude fruit extract. Appearance of a pink colouration showed that flavonoids were present in the extract.

Saponins
An aliquot (2 mL) of the crude fruit extract was added to 5 mL of distilled water and mixed thoroughly. A few drops of olive oil was added and then mixed. Appearance of lather indicated the presence of saponins.

Tannins
To 0.5 ml of the fruit extract, 10 ml of bromine water was added. Discolouration of the bromine water indicated the presence of tannins.

Steroids
Chloroform and concentrated H_2SO_4 (2 mL each) were added to 5 ml of fruit extract. Appearance of a red colouration in the lower chloroform layer showed the presence of steroids.

Anthraquinones
To 3mL of fruit extract, 3 mL of benzene was added. A few minutes later, 5 mL of ammonia solution was added and the mixture was thoroughly mixed. Presence of a pink, violet or red colouration in ammonia layer showed the presence of anthraquinones.

Alkaloids
One mL of the crude fruit extract was acidified with 1% HCl and the solution was allowed to stand for 2 min. Afterwards, a few drops of Dragendoffs reagents were added to the acidified crude extract and mixed thoroughly. Formation of an orange-brown precipitate showed that alkaloids were present.

Terpenoids
To 5 ml of fruit extract, 2.0 ml of chloroform was added, and the solution was put in a boiling water bath for 1 hour to evaporate. Three mL of concentrated H_2SO_4 was added to the left over and brought to boil. Formation of a grey colour indicated that terpenoids were present.

Glycosides
The Keller-Kiliani test was employed to test for glycosides. To 10 ml of fruit extract, a drop of 2.0% $FeCl_3$, 4 ml of glacial acetic acid solution, and 1ml concentrated H_2SO_4 was also added. Formation of brown ring revealed the presence of cardiac steroidal glycosides.

Carbohydrates
Crude fruit extract was added to boiling Fehling solution, and the appearance of a red coloured precipitate showed that reducing sugars were present. To test for carbohydrates, an aliquot of crude fruit extract was boiled with 2 ml of Benedict's reagent, and the formation of a reddish-brown colour confirmed the presence of carbohydrates.

Quantification of total phenolics
Total phenolic content was determined using the Folin-Ciocalteu (FC) colorimetric method (Waterhouse, 2002). Crude extract was diluted with distilled water using a dilution factor of 10. To 2 ml of diluted extract, 1ml of 10% (v/v) FC reagent was added, and after 7 min, 1 ml of 7.5% sodium carbonate was added to the mixture. The solution was mixed thoroughly and left to incubate for 90 min at 25ºC. Afterwards, the optical density of the solution was measured at 725 nm using UV-Vis Spectrophotometer (Spectrum lab 725s, China) in triplicates. Standard solution of gallic acid (5–500 mg/L) was also prepared using serial dilution and the calibration curve was constructed. Concentration of total phenolic was quantified and expressed as milligram gallic acid equivalent (GAE) per

gram of dry weight of samples.

Quantification of total flavonoids

Total flavonoids content was determined using aluminium chloride ($AlCl_3$) colorimetric method (Chang et al., 2002). Briefly, 1 mL of the extract was added to 0.3 ml of 5% $NaNO_3$ in a 10 ml volumetric flask. After 5 min at quiescence, 0.3 ml of 10% $AlCl_3$ was pipetted into the volumetric flask and reaction was allowed to take place for 1 min. Afterwards, 2 ml of 1 M NaOH was pipetted into the flask and the volume was made up to 10 ml with distilled water. The content of the flask was further mixed and the absorbance was measured at 510 nm using UV-Vis Spectrophotometer (Spectrum lab 725s, China) in triplicates. Various concentrations (5–200 µg/mL) were prepared from the stock solution of quercetin standard (1mg/ml) using serial dilution to obtain the standard calibration curve. Quercetin standard calibration curve was constructed and concentration of total flavonoids in each sample was determined and expressed as milligram quercetin equivalent per gram of dry weight of sample.

DDPH Assay

DDPH scavenging ability of methanolic extract of the fruit samples was measured using the method of Akowuah *et al.* (2005). Briefly, fruit extracts were diluted with a factor of 10. To 1 mL of the diluted extract, 1 mL of 100 µM DPPH in methanolic solution was added. The resultant solution was thoroughly mixed and incubated for 30 min at 25 ºC. Thereafter, absorbance was measured at 517 nm in triplicates. The percent inhibition of DPPH was then calculated using the formula below:

% DPPH inhibition = $[(A_0 - A_1)/A_0] \times 100$,
where A_0 = absorbance of the control
and A_1 = absorbance of the test extracts.

Ferric Reducing Antioxidant Power Assay

Ferric Reducing Antioxidant Power (FRAP) of the fruit extracts was determined according to Benzie and Strain (1999). To prepare the FRAP reagent, 30 mM acetate buffer of pH 3.6, 10 mM TPTZ solution and 20 mM ferric chloride solution were respectively mixed in the ratio 10:1:1 (v/v/v). To 1 mL of the diluted sample extract, 2 mL of FRAP reagent was added and thoroughly mixed. This was incubated at room temperature for 30 min before absorbance was measured at 593 nm in triplicates. A blank solution was prepared by adding 1 mL of distilled water to 2 mL of FRAP reagent. $FeSO_4.7H_20$ at 0.001 M concentration was employed as the standard. Standard curve of absorbance versus Fe^{2+} concentrations was constructed and FRAP value of sample, expressed in µM, was obtained using this formula:

$$\frac{[\text{Abs (sample)} \times \text{FRAP value of standard (µM)}]}{\text{Abs (standard)}}$$

Data analysis

Data were analysed using IBM SPSS version 20.0. The results were expressed as means ± standard deviation (SD) of three replicates. Mean values obtained were compared using one-way analysis of variance (ANOVA) at $p<0.05$. Duncan's Multiple-range test was employed to separate significant means at $p<0.05$.

Results and Discussions

Preliminary screening for phytochemicals

Phytochemicals are naturally-occurring secondary metabolites in plant and are very important for the well-being of humans (Bazzano *et al.,* 2002). They are reported to act as antioxidants and anti-inflammatory agents, and play significant roles in combating free radicals, hence reducing the risk of degenerative and oxidative stress induced diseases. The qualitative screening of the

phytochemical constituents of African bush mango seeds and pulp, soursop seeds and pulp and pineapple peels and pulp are presented in Table 1.

All fruit samples were found to test positive for phenolics, flavonoids, alkaloids, tannins, saponins, cardiac glycosides, and carbohydrates. This observation was in consonance with reports of several other researchers who also reported the presence of these secondary metabolites in pineapple, soursop and African bush mango fruits (Wolfe and Ijeoma, 2010; Matos *et al.,* 2009; Arogba, 2014; Alshammaa, 2016 and Don, 2018).

Table 1: Preliminary screening for phytochemicals in the edible and non-edible parts of the selected tropical fruits

Phytochemical	African bush mango		Soursop		Pine-apple	
	Seed	Pulp	Seed	Pulp	Peel	Pulp
Phenolics	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Steroids	-	+	+	+	+	+
Anthraquinones	-	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+

Note: (+) implies presence; (-) implies absence

The presence of phenolics, flavonoids, saponins, alkaloids, tannins and cardiac glycosides have been previously reported in the various tissue parts of African bush mango, soursop and pineapple from various countries, however, data on various tissues of these tropical fruits are scanty in Nigeria. Soursop seeds and pulps were reported to test positive for phenolics, flavonoids, alkaloids, tannins, saponins, terpenoids and cardiac glycosides (Onyechi *et al*., 2012; Padmini *et al.,* 2014; Gavamukulya *et al.,* 2014; Vinothini and Growther, 2016; Agu and Okolie, 2017). The reports of Gunwantrao *et al.* (2016) showed that anthraquinones, phenolics, flavonoids, steroids, tannins and saponins exist in pineapple peel extracts, while Kaushik and Kundu (2018) reported for the presence of tannins, cardiac glycosides, phenolics, flavonoids and steroids in pineapple pulps. Terpenoids exist in the relevant

Table 2: Total phenol and flavonoid contents of the selected tropical fruits

	African bush mango		Soursop		Pineapple	
	Seed	Pulp	Seed	Pulp	Peel	Pulp
Total phenolic (mg GAE/g dw)	62.7±0.28^{d}	61.5±0.12^{d}	98.4±0.35^{c}	550.7±0.33^{a}	103.17±0.40^{b}	97.18±0.35^{e}
Total flavonoid (mg QE/g dw)	45.3±2.23^{e}	43.1±2.91^{e}	83.8±2.66^{c}	224.7±3.48^{a}	99.2± 1.25^{b}	67.8± 1.32^{d}

Note: Values reported are mean ± SD of triplicates. Values with the same letters on the same row are not statistically different ($p<0.05$). GAE = Gallic Acid Equivalent; dw=dry weight; QE = Quercetin Equivalent

Table 3: Antioxidant activity of tissues of the selected tropical fruits

	African bush mango		Soursop		Pineapple	
	Seed	Pulp	Seed	Pulp	Peel	Pulp
DPPH (% inhibition)	53.4±0.34^{d}	52.1±1.75^{d}	75.3±0.25^{b}	84.3±0.26^{a}	73.6±0.41^{b}	65.4±0.74^{c}
FRAP (μM)	8.21±3.68^{c}	7.19±1.83^{c}	28.74±0.31^{a}	29.15±0.20^{a}	28.59±0.18^{a}	13.57±2.58^{b}

Note: All values are expressed as mean ± SD of triplicates. Values with different letters on the same row are significantly different ($p<0.05$).

tissues of all tested fruits with the exception of pineapple pulp while the presence of steroids and anthraquinone was observed in pineapple peels only.

The existence of these bioactive compounds in the tissues suggests the potential health and medicinal roles played by these fruits. For instance, tannins are generally known to possess the ability to fight against free radicals and also have antimicrobial, anti-parasitic and analgesic potentials (Starec *et al.,* 1988). In addition, saponins also possess antioxidant activity, anti-carcinogenic and immuno-stimulant properties (Rao and Sunk, 1995). Usunomena (2012) has reported the importance of cardiac glycosides in fruits in relation to the management of cardiovascular diseases. Phenolics and flavonoids detected in the fruits are known to be strong antioxidants and have been proposed to contribute significantly to the pharmacological benefits attributable to several fruits in complementary medicine (Dembitsky *et al*., 2011).

Total phenolic and flavonoid contents

The total phenolic content of the fruits ranged from 61.5±0.12 mg GAE/g dw in African bush mango pulp to 550.7±0.33 mg GAE/g dw in soursop pulp (Table 2). Soursop pulp was also observed to have the highest flavonoid content (224.7±3.48 mg QE/g dw) while African bush mango pulp (43.1±2.91 mg QE/g dw) had the lowest content. No marked difference was observed between the seeds and pulp of African bush mango for total phenolics and flavonoids, respectively. Soursop pulp contained significantly ($p<0.05$) higher total phenolic and flavonoid contents than the seeds. This observation was consistent with the reports of Adefegha *et al.* (2015), who also reported similar values for the total phenolics and flavonoids in soursop seeds and pulp, with higher contents of these secondary metabolites in the pulp than the seeds.

Generally, the concentration of phenolics and flavonoids in the studied fruit peels, seeds, and pulp are considerably high. This suggests that the fruit pulps might be beneficial for human consumption to fight oxidative stress induced diseases. In addition, the peels and the seeds that might not be consumed directly might find application in the nutraceutical and food industries as natural antioxidants. The tissue differential contents of the metabolites could be adduced to the fact that the pericarp and pulp are more exposed to UV light from the sunlight, a form of environmental stress factor (Soumaya *et al.,* 2013), that is known to promote the synthesis of bioactive substances

in plants. Chanwitheesuk *et al.* (2005) proposed that stress factors could aggravate the production of phenolic compounds in plants to prevent oxidative damage that may affect plant cellular structures. The seeds have less exposure to UV light from sunlight because they are well protected by the pulp and epicarp. However, this was not obtainable in African bush mango, which may be as a result of variations in the species type of the fruits. African bush mango has a hard mesocarp compared to soursop and pineapple.

In this study, total phenolic and flavonoid contents of pineapple peels was observed to be significantly higher ($p<0.05$) than the pulp. Hossain and Rahman (2011) have also previously reported similar observations. Total phenolic content of pineapple obtained in this study was slightly higher than the values reported in other studies (Hossain and Rahman, 2011; Gunwantrao *et al.,* 2016; Kaushik and Kundu, 2018). This difference could be due to variation in postharvest conditions, handling, geographical location (Liu et al., 2018) as well as the different analytical methods employed.

Total antioxidant activity

Total antioxidant activity of the studied fruits is reported in Table 3. Soursop pulp had the highest percent inhibition of DPPH free radical (84.3% ± 0.26), while African bush mango pulp exhibited the lowest inhibition potential (52.1% ± 1.75). Also, FRAP values ranged from 7.19 ± 1.83 µM in African bush mango pulp to 28.59 ± 0.18 µM in pineapple peels.

In the present study, fruits with higher total phenolic and flavonoid contents were observed to possess higher antioxidant potential as defined by DPPH and FRAP assays. Tomas-Barberan and Espin (2001) postulated that bioactive constituents and their structure mostly determine the antioxidant capacity and efficacy of biological activities of a fruit. Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups (Panche et al., 2016). Phenolic and flavonoid molecules are essential antioxidants that possess ideal structural features capable of scavenging and neutralizing free radicals because of their ability to donate hydrogen atoms to free radicals (Chithiraikumar et al., 2017).

Although, positive association between antioxidant capacity and phenolic contents of plants have been reported in some studies (Chanwitheesuk et al., 2005; Ghasemzadeh et al., 2010; Padmini et al., 2014), there is still the need to further characterise bioactive constituents of these fruits to determine the phenolic composition responsible for the specific antioxidant and biological activities. For instance, Shahidi & Naczk (1995) indicated that phenolic compounds with ortho- and para-dihydroxylation or a hydroxy and a methoxy group have higher antioxidant capacity and biological activity than the simple phenols. In addition, the interactions of all the bioactive substances present in a crude fruit extract cannot be ruled out for synergistic effects (Liu, 2003).

Conclusion

Seeds and pulp of African bush mango and soursop as well as the peels and pulp of pineapple fruits are good sources of natural antioxidants and bioactive secondary metabolites. The various tissues of these fruits contain significant amounts of phenolics and flavonoids with known high antioxidant activity, beneficial in combating inflammation and oxidative stress-induced diseases. In addition, they could also serve as good low-cost alternatives for functional foods or natural antioxidant ingredients, particularly in the food processing industries. Further-

more, it is important to further isolate and identify the antioxidant components and study their mechanism of action for enhanced understanding about their ability to control diseases and improve quality of life.

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