

Antioxidant activity, total phenols and phytochemical constituents of four underutilised tropical fruits

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Article history

Received: 15 January 2014

Received in revised form:

16 June 2014

Accepted: 25 June 2014

Keywords

Antioxidant activity

Phytochemicals

Total phenols

Underutilised fruits

Abstract

The antioxidant activity, total phenols content and phytochemical constituents of edible portions of four underutilised tropical fruits; *Irvingia gabonensis* (African mango), *Artocarpus altilis* (breadfruit), *Annona muricata* (soursop) and *Annona squamosa* (sweetsop) were determined. Extracts of fruit samples were screened for selected phytochemicals using standard methods whereas the total phenols content and antioxidant activity (by free radical scavenging action) was determined by the Folin-Ciocalteu method and DPPH assay, respectively. The antioxidant activities were high with values ranging from 63% inhibition (breadfruit) to 78% inhibition (African mango pulp). African mango seeds had the highest total phenols content (20.96 mg GAE /100g). Tannins, triterpenoids, saponins, sterols, cardiac glycosides, flavanoids and coumarins were detected in most of the fruit extracts. The study revealed that these underutilised tropical fruits have considerable free radical scavenging (antioxidant) activity and an array of phytochemicals necessary to significantly impact health of consumers.

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Introduction

Highly reactive free radicals present in biological systems arise from cell metabolic activities (prominently, oxidation) (Pisoschi *et al.*, 2009) and external factors such as tobacco smoke, UV radiation and other environmental pollutants (Murillo *et al.*, 2012). These initiate degenerative reactions in biological systems resulting in secondary damage to biological molecules especially the biomembranes such as proteins, lipids and nucleic acids (Pisoschi *et al.*, 2009). Many human disorders viz., coronary heart diseases, atherosclerosis, aging, some cancers, chronic fatigue and Alzheimer's disease have been attributed to the degenerative reactions of these oxidising agents (Liu, 2003; Sochor *et al.*, 2010).

Biological systems are in equilibrium when the effects of these reactive species are offset by the activity of inherent antioxidants which may be enzymes or non-enzymatic compounds. Thus, the antioxidant activity of a system gives a measure of its protective abilities against degenerative/oxidative reactions induced by oxidising agents (Sochor *et al.*, 2010). However, most human cells unlike plant cells do not generate adequate amounts of antioxidants to protect them against oxidative reactions of the produced reactive species (Pisoschi *et al.*, 2009)

thus, the need to look for more dietary sources of antioxidants (Liu, 2003). The demand is more pronounced in this era of increased number of non-communicable diseases (NCDs) worldwide. This is especially so for developing nations where the rise in the NCDs is being battled alongside the existing menace of infectious diseases (Anonymous, 2006; Hall *et al.*, 2009).

Fruits represent one such source of dietary antioxidants (Liu, 2003; Kolar *et al.*, 2011) thereby necessitating the call for their increased consumption in recent times (Hall *et al.*, 2009). The total antioxidant (free radical scavenging) activity of fruits is mainly attributed to the additive and synergistic effects of inherent phytochemicals notably, the phenolic compounds (Liu, 2003; Cartea *et al.*, 2011). In addition to their antioxidant activity, phytochemicals also impart other peculiar beneficial toxicological/ pharmacological effects in humans. Thus, a measure of the free radical scavenging activity and phytochemical composition of fruits gives an indication of the potential health benefits to be derived from their consumption (Liu, 2003; Pisoschi *et al.*, 2009; Vera de Rosso, 2013). It is known that natural sources of antioxidants such as fruits and vegetables are more advantageous to health than the synthetic counterparts/supplements (Liu, 2003).

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Despite extensive research on the antioxidant activity and phytochemical composition of most common fruits, little is known about the many tropical underutilised fruits in developing nations. Ghana faces a similar challenge with its myriad of underutilised tree fruits (Abebrese *et al.*, 2007). In a bid to contribute to measures aimed at emphasising diets in preventive health-care (Hall *et al.*, 2009), the edible portions of local varieties of four such underutilised fruits, *Annona squamosa* (sweetsop pulp), *Annona muricata* (soursop pulp), *Irvingia gabonensis* (African mango - pulp and seeds) and *Artocarpus altilis* (breadfruit pulp) were studied. The fruit samples were screened for the presence of major phytochemicals and their total antioxidant activity determined to assess their potential protective benefits against degenerative reactions induced by free radicals. The obtained data also provides baseline information on the fruits for further research works.

Materials and Methods

Source of materials

Irvingia gabonensis and *Artocarpus altilis* fruits were obtained from the Kwame Nkrumah University of Science and Technology (KNUST) campus whereas those of *Annona muricata* and *Annona squamosa* were purchased from local markets in Kumasi and Accra, respectively. All chemicals used were analytical grade reagents.

Sampling and preliminary sample preparation

Fruits were taken to the laboratory, sorted, washed under running water, dried and then weighed. In all, sixty African mangoes, five breadfruits, ten soursop and twenty sweetsop fruits with average weights of 195 g, 1267 g, 415 g and 177 g, respectively were selected for use. The samples were peeled and where applicable, the seeds removed from the pulp. The pulp of African mango and breadfruit were reduced to a thickness of 0.2 mm with a laboratory slicer (Model ART NO.S-727, China) while the African mango seeds were reduced to a similar thickness with a stainless steel knife.

Prepared samples were bagged in pre-weighed zip-lock pouches and then frozen for 2 - 5 days at -20°C prior to freeze-drying. The stored samples were freeze-dried in a vacuum freeze-drier (Model YK - 118 - 50, True Ten Industrial Co. Ltd, Taiwan) for 44 h prior to the various determinations. The freeze-dried samples were then milled (Thomas scientific mini-miller; Model 3383-L70), sieved with an impact lab test sieve of pore-size 400/425 µm (Model BS410 - 1:2000) bagged in zip-lock pouches

and then stored at -20°C prior to analysis.

Determination of antioxidant activity (AOA)

The freeze-dried samples were weighed into 5 mg portions using a weighing balance (Mettler Toledo.XP 105 Delta Range) into separate eppendorf tubes. 1.0 ml of methanol was then added to each sample, vortexed (Vortex V-1 Plus BM BIO) and then sonicated (Astrason TM Ultrasonic Cleaner Model) for 2 h. The solution was decanted and the supernatant hereafter referred to as fruit extract, used for the AOA determinations by the DPPH assay according to Ghasemi *et al.* (2009). The DPPH assay is fairly simple and quick to use and has been widely used in assessing the AOA of foods (Pisoschi *et al.*, 2009; Sharma and Bhat, 2009). It also has the ability to efficiently measure the total AOA of the substrate (under study) without necessarily being specific to any particular antioxidant present (Prakash *et al.*, 2001; Sharma and Bhat, 2009). The results were expressed as percentage inhibition of the DPPH radical which was calculated according to the equation:

$$\% \text{ DPPH} = \frac{\text{Absorbance of blank} - \text{Absorbance of extract}}{\text{Absorbance of blank}} \times 100$$

Where, Absorbance of blank is the absorbance of DPPH solution without extracts. All determinations were carried out at least in duplicates.

Phytochemical screening

The phytochemical screening was done following the standard testing protocols as previously reported (Trease and Evans, 2002; Onike, 2010; Tiwari *et al.*, 2011).

Determination of total phenolics

Sample extraction was performed as described for the AOA determination above. The total phenolic content of the methanolic extract of each fruit was determined using the Folin-Ciocalteu method described by Waterhouse (2002). The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per 100 g of fruit. All determinations were carried out at least in duplicates.

Statistical analysis

The results obtained were analyzed statistically using SPSS (v.20, IBM SPSS Statistics, US) The mean total phenol content and antioxidant activity of the four fruits samples were compared using one-way ANOVA followed by LSD post hoc multiple comparisons. Statistical significant difference was performed at $\alpha = 0.05$

Table 1. Phytochemical constituents of the edible portions of four underutilised tropical fruits

Phytochemicals	Fruits				
	AMP	AMS	Breadfruit	Soursop	Sweetsop
Tannins	+	+	+	+	+
Saponins	+	+	+	+	-
Sterols	+	+	+	-	-
Alkaloids	-	-	-	-	-
Glycosides	+	+	+	+	+
-Cyanogenic	-	-	-	-	-
-Cardiac	-	+	-	+	+
Flavonoids	+	+	-	+	-
Coumarins	-	-	+	+	-
Triterpenoids	+	+	+	-	+

(+) implies presence; (-) implies absence

Results and Discussion

Antioxidant activity (AOA) of fruit samples

The studied fruits showed fairly high free radical scavenging (antioxidant) activities against DPPH compared to the activity of the synthetic antioxidant, BHT (91.76%) (Figure 1). African mango pulp and sweetsop had free radical scavenging activities of 75.45% and 68.10% inhibition, respectively. African mango seeds, Soursop, and breadfruit also had free radical scavenging activities of 60.56%, 75.39% and 61.93% inhibition, respectively. These findings corroborate previous reports by Agbor *et al.* (2011), Kolar *et al.* (2011) and Murillo *et al.* (2012) for Cameroonian, Indian and Panama varieties of soursop, breadfruit and African mango seeds, respectively. The obtained AOA of the underutilised fruits also compared favourably with that of apple pulp (69.1% inhibition) (Leontowicz *et al.*, 2003) as well as being superior in terms of their AOA to some other common fruits such as banana with AOA of 26.55% - 29.38% inhibition for two varieties (Fatemeh *et al.*, 2012) and orange with 47.5% - 49.2% inhibition (Klimczak *et al.*, 2006). Rawat *et al.* (2010) and Murillo *et al.* (2012) proposed the AOA as an important parameter in measuring the health and nutritional potential of fruits. A review by Liu (2003) also confirmed this and suggested the different mechanisms of antioxidants (prominently, scavenging of free radicals) to be responsible for the inverse relations between their consumption and many non-communicable diseases including coronary heart diseases, diabetes and some cancers. Thus, the fairly high free-radical scavenging capacity (AOA) of the edible portions of the studied underutilised fruits is desirable in seeking optimum health of the populace.

Phytochemical constituents

The detected phytochemicals of fruit extracts are shown in Table 1. On the whole, tannins and glycosides were detected in all the samples while saponins were detected in all except sweetsop. Triterpenoids were

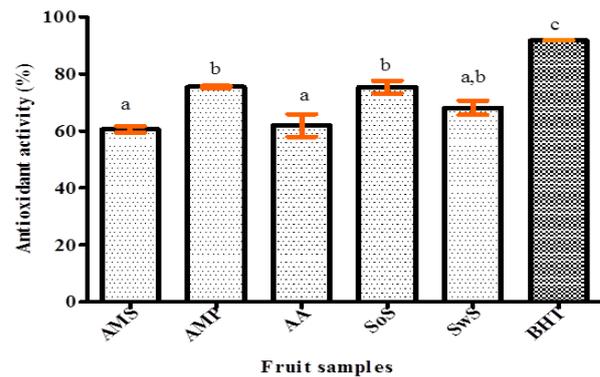


Figure 1. Free-radical scavenging activity of fruit samples against DPPH. Plots with different alphabets indicated on top are statistically different ($P < 0.05$). AMP- African mango pulp; AMS- African mango seeds; BF- breadfruit; SoS- soursop; SwS- sweetsop

also detected in all the samples except in soursop. Sterols and flavanoids were detected in three out of the five samples whereas coumarins were present in only breadfruit and soursop. Alkaloids were not detected in any of the fruit extracts.

Tannins are acclaimed for their free-radical scavenging activity, antimicrobial, antiviral and anti-inflammatory properties (Akiyama *et al.*, 2001). Thus, the detection of tannins in all the extracts indicates the potential health benefits of the fruits. It is worth mentioning that the tannin content of these fruits when ripe is just adequate to prevent the astringent and bitter taste imparted by tannins to some plant-based foods (Adekunle and Oyerinde, 2004; Etebu, 2012).

Saponins were detected in African mango (seeds and pulp), soursop and breadfruit in harmony with reports by Wolfe *et al.* (2010), Etebu (2012) and Onyechi *et al.* (2012) respectively. Saponins are common in most plants and have been postulated to have a wide range of biological activity including antioxidant, anticarcinogenic as well as having immunostimulant properties thereby exhibiting the potential to cure a number of diseases (Trease and Evans, 2002). It is noteworthy that although Francis *et al.* (2002) reported adverse effects of some types

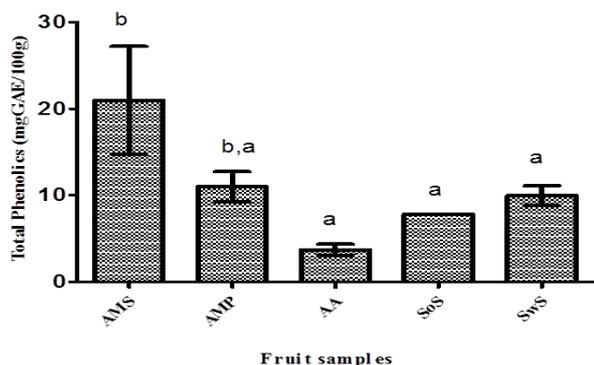


Figure 2. The total phenolic contents (mg GAE/ 100 g) of fruit samples. Plots with different alphabets indicated on top are statistically different ($P < 0.05$). AMP- African mango pulp; AMS- African mango seeds; BF- breadfruit; SoS- soursop; SwS- sweetsop

of saponins on fishes (and other aquatic lives), the researchers also indicated the associated health beneficial properties (to humans) of those found in edible fruits thereby eroding any cause for alarm in the consumption of these fruits.

The general test for glycosides gave a positive inference for all the samples. Glycosides collectively represent compounds with a pharmacologically active genin and at least one reducing sugar. Specific tests for cyanogenic glycosides had negative results for all the samples. It has however been reported to be present in minute amounts in sweetsop (Coronel, 1986). The absence of cyanogenic glycosides in the fruits is desired because of the toxicity associated with the intake of this compound. However, Onike (2010) suggested relaxant and calming effects by the chemical on the heart and muscles when it is consumed in small doses.

Tests for cardiac glycosides gave positive inferences for African mango seeds, soursop and sweetsop. Usunomena (2012) reported the importance of cardiac glycosides in treating congestive heart failure thus its presence in these fruits is much desired. Moreover, the findings support a review by Dembitsky *et al.* (2011) in which the researchers suggested the potential of soursop and sweetsop in the treatment of cardiac-related ailments (as used in folkloric medicine) by analysing their cardiac glycoside composition.

Flavanoids were detected in African mango (pulp and seeds) and soursop. The findings corroborates earlier detections for some Nigerian varieties by Wolfe *et al.* (2010) for African mango seeds, Etebu (2012) for African mango pulp and Onyechi *et al.* (2012) for soursop. Flavanoids are acclaimed for their antioxidant and antimicrobial activity (Dembitsky *et al.*, 2011). The absence of flavanoids in the studied breadfruit and sweetsop samples is in agreement with

reports of non-detection by Ragone (2011) for some breadfruit varieties in the Pacific Islands and EFSA (2009) for sweetsop from the Philippines.

A positive inference for coumarins was obtained for soursop and breadfruit. Adewole and Ojewole (2008) reported the presence of coumarins in soursop but there is dearth of data on its presence in breadfruit. Coumarins were not detected in sweetsop and African mango (both pulp and seeds) in the study. Coumarins are not usually assessed in the phytochemical analysis of most fruits (EFSA, 2009; Wolfe *et al.*, 2010; Etebu, 2012) resulting in a dearth of information on them. According to Trease and Evans (2002) however, coumarins are anti-stress compounds that are postulated to have antiviral (including anti- HIV) activity. They are also employed as oral-anticoagulants – an essential agent in the treatment of arterial thrombosis. Thus, their detection in breadfruit and soursop shows added potential benefits of the fruits.

The presence of sterols as observed in African mango (pulp; seeds) and breadfruit is in accordance with a report by Amarasinghe *et al.* (2008). It was not detected in soursop contrary to a report by Adewole and Ojewole (2008) and also not detected in sweetsop. Awad and Fink (2000) suggested strong inverse relations between intake of plant sterol and risk of some cancers. This was corroborated by De Stafani *et al.* (2000) in a controlled case-study that showed the consumption of plant sterols to be inversely proportional to the occurrence of stomach cancer. The detection of this plant chemical in breadfruit and African mango (pulp and seeds) therefore, is desirable.

The general test for triterpenoids on the other hand, gave positive inferences for all the samples except for soursop. The detection of the chemical in breadfruit and African mango seeds has been reported by Wang *et al.* (2006) and Wolfe *et al.* (2010) but data on its presence in African mango pulp and sweetsop is scarce although it was detected in this study. Sweetsop which tested positive to triterpenoids had a negative inference for the sterol test. This corroborates an assertion by Trease and Evans (2002) that all sterols are triterpenoids but the vice-versa is untrue. Varadharajan *et al.* (2012) reported wound healing properties responsible for wound contraction and epithelialisation by triterpenoids – a potential that makes their presence in foods desirable.

All the fruit extracts showed negative inferences to the test for alkaloids. Alkaloids have not been detected in sweetsop (EFSA, 2009) and breadfruit (Ragone, 2011) but have been detected in African mango seeds and pulp (Etebu, 2012) as well as in

the mesocarp of soursop (EFSA, 2009). However, Etebu (2012) showed a decline in alkaloid content of African mango mesocarp with increasing storage duration suggesting the need for the analysis to be done on fresh samples for an adequate assessment. On the whole, the array of phytochemicals detected in the extracts of the underutilised fruits, further indicate their potential contribution to health especially in areas where they are abundant.

Total phenolic compounds

Quantification of the total soluble phenols detected is shown in Figure 2. The values ranged from 3.71 mg GAE/100g for breadfruit to 20.95 mg GAE/100g for African mango seeds. However, limited studies on these underutilised fruits viz., Agbor *et al.* (2005) (on African mango seeds from Cameroun), Kolar *et al.* (2011) on some Indian varieties of breadfruit and Vera de Rosso (2013) on sweetsop varieties from Brazil reported comparatively higher phenolic contents than the obtained values for this study.

The variations may be attributed to varietal differences resulting from different climatic and pre-harvest conditions as well as genotypic differences as suggested by Rawat (2011) who reported significant source specific variations in the phenolic contents of the same species of some fruits. Agbor *et al.* (2005) and Deng *et al.* (2012) also reported phenolic constituents of plant foods to be either hydrophilic and/ or lipophilic - suggesting their analysis to be invariably influenced by the solvent employed, extraction methods and length of the extraction.

These factors may also have contributed to the fairly low content obtained indicating the need for an in-depth study of the nature of the inherent phenolics of the studied fruits in-order to adequately quantify them. In spite of this shortfall, the phenol content of the African mango seed (20.95 mg GAE/100g) is comparable to that of pineapple with 20.20 – 26.20 mg GAE/100g and mango with 20.0 – 40.0 mg GAE/100g (Nixwell *et al.*, 2013) further indicating the potential of the underutilised fruits as alternate dietary antioxidant sources for optimal health.

Phenolic compounds being part of the total phytochemical composition of plants are acclaimed for their high free radical scavenging ability in addition to having other disease prevention potential such as being antimicrobial, antiviral and anti-inflammatory agents (Gui-Fang *et al.*, 2012; Oskana *et al.*, 2012). However, taken as a whole, there was non-significant correlation ($P < 0.05$) between the total soluble phenolics of the fruits and their AOA. This supports the suggestion by Murillo *et al.* (2012) that the total phenolics of different fruit species

have different degrees of contributions to their AOA and the relationship observed in one, cannot be generalised for all others.

Conclusion

The studied underutilised tropical fruits had fairly high antioxidant (free radical scavenging) activity. Generally, the fruit extracts showed positive inferences to the major phytochemicals tested except for the presence of alkaloids. These include the presence of coumarins in breadfruit and soursop, cardiac glycosides in soursop, sweetsop and African mango seeds as well as triterpenoids in African mango pulp and sweetsop. Thus, there are potential health benefits to be derived from the incorporation of these fruits into diets and this indicates the need for their exploitation in seeking optimum health of the populace.

Acknowledgement

The authors are grateful to the Chemical Pathology Department of Noguchi Memorial Institute for Medical Research, Ghana, for the technical support received in the determination of total phenols and antioxidant activity.

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