International Food Research Journal 23(Suppl): S179-S185 (December 2016)

Journal homepage: http://www.ifrj.upm.edu.my

Antioxidant potential, phytochemical and nutrient compositions of Nigerian hog plum (*Spondias mombin*) seed kernel as a new food source

^{1,3*}Esua, O.J., ²Makinde, O.O., ²Arueya, G.L. and ³Chin, N.L.

¹Department of Agricultural and Food Engineering, Faculty of Engineering, University of Uyo. P.M.B 1017, Uyo, 520101, Nigeria ²Department of Food Technology, Faculty of Technology, University of Ibadan, Ibadan, 200284, Nigeria

> ³Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia

Article history

Received: 16 June 2016 Received in revised form: 11 August 2016 Accepted: 11 August 2016

Keywords

Spondias mombin Proximate Mineral Phytochemical Anti-nutritional

<u>Abstract</u>

The Nigerian hog plum (Spondias mombin) seed kernel was studied for its possible antioxidative potential and its proximate and nutrient compositions measured. The fruits were submitted to washing, pulp removal and drying to facilitate fibre separation from the seed kernel. Seeds were Milled to obtain samples, while analysis was carried out using standard Association of Analytical Communities (AOAC) methods and phytochemical assays. The seed kernel contains carbohydrate (40.56%), ash (8.09%), crude fibre (31.86%), moisture content (8.48%), crude protein (7.73%), crude fat (3.28%), calcium (1317 mg/kg), iron (839.08 mg/ kg), magnesium (494.71 mg/kg), manganese (17.93 mg/kg), zinc (15.27 mg/kg), and copper (7.68 mg/kg). Phytochemical analyses indicated the presence of tannins (0.06%) and phytate (0.0022%), but did not indicate the presence of flavonoids, oxalate and saponins. The free radical scavenging activity against the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was at the level of 15.09%, while total antioxidant capacity and total phenolic content were 856.7±5.84 mgAAE/100g and 573.32±11.5 mgGAE/100g respectively. The seed kernel of Spondias mombin is a potential source of nutritious food, good anti-anaemic and anti-diabetic agent as a result of iron and zinc content, and potential chemo-protective agent with the ability to offer possible protection against the activities of the toxic Reactive Oxygen Species (ROS) from the results of total phenolic content, antioxidant and free radical scavenging activities.

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Introduction

Spondias mombin is a fructiferous tree which belongs to the Spondias genus of the flowering plant family, Anacardiaceae, native to the lowland moist forest of the Amazon (Adedokun et al., 2010; Bicas et al., 2011). The distribution of the plant spans all of tropical America, Brazil, Nigeria, West indies and other tropical rain forests of the world (Duvall, 2006; Adedokun et al., 2010; Bicas et al., 2011; Mattieto and Matto, 2011). The various common names include Bala (Costa Rica), Jobito (Panama), Jobo blanco (Colombia), Jobo corronchoso (Venezuela), Hoeboe (Surinam), Acaiba, Caja, Pau da tapera (Brazil), Ubo (Peru), and Hobo (Mexico) (Ayoka et al., 2008); plum, Jocote, Red Mombin, Purple Mombin, Hog Plum, Sineguela, and Siriguela (Olayemi et al., 2013). It is identified with different names in Nigeria; the Edo call the fruit 'oheeghe', while the Yoruba calls the fruit 'iyeye' and the tree 'akika'. The tree is also called 'nsukakara' by the Efik, 'tsadar masar' by the Hausa, 'ijikara', 'ogogo', 'ngwu' or 'ungwu' by the Igbo, 'aginiran' by the Ijaw and 'kakka' by the Tiv (Fadimu *et al.*, 2012). It is a single stemmed tree which grows in the rainforest and coastal areas as high as 20 m with a moderate canopy and scattering crown. The fruit is one seeded, round, oblong, or ovoid drupes with various weights and sizes often ranging from 4 - 43 g and from 20 to 50 mm respectively (Maldonado-Astuillo *et al.*, 2014). It is similar to the temperate plum, skin turns yellow to orange once ripened and possess a pleasant acid taste and aroma (Bicas *et al.*, 2011; Fadimu *et al.*, 2012; Olayemi *et al.*, 2013).

Data from various sources have identified increased consumption of the fruit of *Spondias mombin* as food in raw and processed form as a result of great taste (which ranges from sour to sour sweet), good levels of minerals, vitamins, low levels of lipids and reducing sugars (Ayoka *et al.*, 2008; Mattieto and Matto, 2011; Akther *et al.*, 2012; Olayemi *et al.*, 2013; Maldonado-Astuillo *et al.*, 2014). Recent studies also suggest that the fruit of *Spondias mombin* is a rich source of antioxidants and phenols, minerals, phytonutrients, organic acids, phytosterols, terpenoids, and carotenoids (Arif and Fareed, 2010; Tiburski *et al.*, 2011; Olayiwola *et al.*, 2013). It has been argued that different parts of the plant are known for their diverse ethnopharmacological use and anthropogenic tendencies (Fadimu *et al.*, 2012; Fadimu *et al.*, 2014).

Findings have established it as a potential source of highly nutritious feed stuff, folk medicine and phytomedicine as a result of its aromatic, astringent and refrigerant nature (Ayoka *et al.*, 2006; Njoku and Akumefula, 2007; Ayoka *et al.*, 2008; Arif and Fareed, 2010; Igwe *et al.*, 2010). The tree exudes gum used as glue; root and bark decoctions are used as purgatives and remedies for diarrhea, dysentery and haemorrhoids (Ayoka *et al.*, 2008); while leaves extract have been reported to have anxiolytic (Ayoka *et al.*, 2005), anathematic, sedative, antiepileptic and antipsychotic (Ayoka *et al.*, 2006), hypoglycaemic (Fred-Jaiyesimi *et al.*, 2010), in vitro antioxidant (Akinmoladun *et al.*, 2010), and antimicrobial (Aromolaran and Badejo, 2014) effects.

With increasing consumption of the *Spondias mombin* fruit, considerable attention is required for analysis of its nutrients and natural compounds. Although considerable amount of literature exists on the importance and usefulness of different parts of the plant, there is little information about the seed kernel which is usually disposed. This study seeks to investigate its possible antioxidant potential, proximate and nutrient compositions so as to unravel new information and warrant its use.

Materials and Method

Sample material and preparation

Fresh fruits of Hog plum were obtained from individual trees at the same harvesting period at the premises of Faculty of Technology and Nnamdi Azikiwe Hall, and identified in the department of Food Science and Technology in University of Ibadan, Nigeria. The fruits were washed, pulp removed and dried at 71°C for 12 hours to facilitate fibre removal from the seed kernel. The seeds were milled to obtain required experimental samples, while analysis was carried out at the Nigerian Institute of Science and Laboratory Technology (NISLT), Ibadan.

Determination of nutritional attributes

The proximate analysis of the seed kernel was

assessed using standard methods of Association of Analytical Communities, AOAC (2000). Moisture content was determined in hot air oven (AOAC method 925.45), ash content by dry ashing (AOAC method 938.08), and protein content by the Kjeldahl method (AOAC method 991.20). A protein conversion factor of 6.25 was used to calculate the percentage of protein from nitrogen determined by the Kjeldahl method. Crude fibre was determined by enzymatic gravimetric method and fat content determined by ether extraction, AOAC methods 985.29 and 989.05 respectively. The carbohydrate content was determined by subtraction of the sum of the values of protein, moisture, fibre, fat and ash from 100%. Minerals including calcium, magnesium, iron, manganese, copper and zinc were analysed by acid digestion (AOAC method 984.2 and 999.10) and read using atomic spectrophotometer (Model 210/211 VGP, Buck Scientific Inc., Connecticut-USA).

Phytochemical contents

The oxalate content was determined by digesting 2 g of sample with 10ml 6 M HCl for 1hr following methods of Adeniyi *et al.* (2009). Spectrophotometric method was used to determine saponin, tannin acid and phytate contents following methods of Brunner (1984), Griffiths and Jones (1977), and Griffith and Thomas (1981) respectively. Saponin content was read at a wavelength of 380 nm, tannin acid solution colour development was read at 760 nm while titration of phytate with ferric chloride solution was read at 519 nm. The flavonoids content was determined using 2 M HCl using the method of Harborne (1996).

Antioxidant activity

The antioxidant activity of the Spondias mombin seed kernel was evaluated through its total phenolic assay, phosphomolydenum assay and free radical scavenging activity. The total phenolic content was determined following the Folin-Ciocalteau method of Singleton and Rossi (1965) where absorption was read at 765 nm and expressed as milligram of gallic acid equivalent (GAE) per gram. The phosphomolydenum assay was regarded as the total antioxidant capacity where phosphomolybdenum complex green formation was measured at absorbance of 695 nm and result was expressed as mg of ascorbic acid equivalents per 100 g (Prieto et al., 1999).

The free radical scavenging activity on 2, 2-dipheyl-1-picrylhydrazyl (DPPH) was determined following method by Ansari *et al.* (2005) where absorbance was measured at 517 nm. Antioxidants react with stable DPPH radical (deep violet colour) and convert it into pale yellow coloured α , α -diphenyl-

 β -picryl hydrazyl, a stable lipophilic free radical which is a measure of non-enzymatic antioxidant activity of plant extracts, quantified by the degree of discolouration (Prasad *et al.*, 2010; Gupta *et al.*, 2011; Deng *et al.*, 2014). The inhibition of DPPH radicals was calculated as scavenging activity using Eqn. 1.

% Inhibition of free radical =
$$100*\left[1-\left(\frac{A-B}{C}\right)\right]$$
 (1)

Where

- A = absorbance of 1.5 ml crude extract solution mixed with equal volume of DPPH solution,
- B = absorbance of 1.5 ml crude extract solution mixed with equal volume of methanol, and
- C = absorbance of blank prepared by mixing 1.5 ml DPPH with equal volume of methanol: water (4:1)

Statistical analysis

The results were expressed as mean \pm standard deviation (SD) from three replicates. Data were evaluated using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Proximate analysis

Table 1 shows the proximate compositions of the seed kernel as compared to its fruit pulp values retrieved from Adepoju (2009) and Tiburski *et al.* (2011). The seed kernel displayed nutrients with highest contents in carbohydrate (41%), followed by fibre (32%), ash (8%), protein (8%), fat (3%) and lastly moisture (8%). The high carbohydrate and fibre content suggests that the flour of the seed kernel could be a good source of energy. The relatively low moisture content of the seed kernel indicates that the seeds and flour prepared from the seeds will have excellent keeping quality.

The total carbohydrate content is comparable to other edible seeds like Cola millenii (37.8%), Annona muricata (47.1%) and Adasonia digitata (52.5%). The Spondias mombin seed submits as potential source of carbohydrates that could be used as a good supplement to the scarce cereal grains in Nigeria as sources of energy in feed formulations. Its higher crude fibre value than most other seeds like the Annona muricata (8.0%), Abelmoschus esculentus (3.5%), Sesemum indicum (2.6%) and Bombax glabra (12.1%) suggests benefits for cholesterol level reduction. Although the crude protein was lower compared to the Abelmoschus esculentus (41.1%), Adasonia digitata (21.8%) and Bombax glabra (34.1%), it is still comparable with the results of other tropical plant seeds like the Sesemum indicum (11.6%) and Annona muricata (2.4%). The relatively low fat content was still higher than other recorded values like Piliostigma thonningii (1.42%). These comparisons were made with references to various works on potential edible seeds of African origin (Onimawo, 2002; Jimoh and Oladiji, 2005; Nkafamiya et al., 2007; Bello et al., 2008; Adelakun et al., 2009; Bamigboye et al., 2010).

Mineral composition

Table 2 lists the mineral elements present in the seed kernel as compared to its fruit pulp. The highest mineral was calcium, followed by magnesium, manganese, iron, copper, and zinc. The seed's mineral composition is consistent with studies by Olayiwola *et al.* (2013); Adepoju (2009) and Tiburski *et al.* (2011), who all confirmed the presence of these minerals in the fruit pulp, but with variations. The variability observed in the amount of these elements obtained for the fruits by these authors could be ascribed to difference in varieties and the geographical location of the plants.

Table 1 Proximate composition of <i>Sponatas mombin</i> seed kernel and fruit pup						
Sample	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Carbohydrate (%)
Seed kernel	8.48 ± 0.03	7.73 ± 0.12	3.28 ± 0.03	31.86 ± 0.08	8.09 ± 0.15	40.56 ± 0.27
^a Fruit pulp	82.30 ± 3.57	2.60 ± 0.04	2.00 ± 0.05	4.20 ± 0.04	1.00 ± 0.02	7.90 ± 0.05
^b Fruit pulp	83.66 ± 0.04	1.06 ± 0.04	0.02 ± 0.05	1.87	0.76 ± 0.01	13.90 ± 0.04

 Table 1 Proximate composition of Spondias mombin seed kernel and fruit pulp

^aAdepoju, 2009; ^bTiburski *et al.* 2011. Values are mean ± standard deviation (n=3)

Table 2 Mineral analysis of Spondias mombin Seed kernel and fruit pulp

		-	-			
Sample	Calcium(mg/kg)	Magnesium(mg/kg)	Manganese(mg/kg)	Iron(mg/kg)	Copper(mg/kg)	Zinc(mg/kg)
Seed kernel	1317.70 ± 21.31	494.71 ± 0.51	17.93 ± 0.10	839.08 ± 1.59	7.68 ± 0.02	15.27 ± 0.02
^a Fruit Pulp	1562.20 ± 0.40	21081.1 ± 282.80	10.80 ± 0.20	145.90 ± 2.20	5.40 ± 0.20	10.80 ± 0.20
^b Fruit Pulp	318.0 ± 4.20	4650.0 ± 212.10	2.0 ± 0.10	32.0 ± 1.40	10.0 ± 1.40	2.0 ± 0.10
° Fruit Pulp	110.38 ± 7.67	150.95 ± 8.63	0.25 ± 0.01	3.27 ± 0.01	1.18 ± 0.37	-

^aOlayiwola et al. 2013; ^bAdepoju, 2009; ^cTiburski et al. 2011. Values are mean ± standard deviation (n=3)

In comparing the *Spondias mombin* seed with the seeds of other commonly consumed fruits and vegetables in Nigeria (Jimoh and Oladiji, 2005; Bello *et al.*, 2008; Kawo *et al.*, 2009; Bamigboye *et al.*, 2010; Lohlum *et al.*, 2010; Edet *et al.*, 2015), the calcium content was significantly higher when compared with *Moringa oleifera* (602 ± 122 mg/kg), *Piliostigma thonningii* (43.11 ± 0.34 mg/kg), and *Lophira lanceolata* (300 ± 15.0 mg/kg), but lower when compared with the *Sesemum indicum* (2811 ± 6.8 mg/kg) and *Cola millenii* (1681.35 ± 30.76 mg/ kg).

Magnesium was lower compared to Allium *cepa* (2320.5 \pm 0.6 mg/kg), *Cola millenii* (3934.01 \pm 87.19 mg/kg) and Lophira lanceolata (820 \pm 26.7 mg/kg), while iron was higher compared to Lophira *lanceolata* (400 \pm 26.7 mg/kg), *Cola millenii* (481.3 \pm 1.41 mg/kg), Sesemum indicum (38.3 \pm 7.5 mg/ kg), and *Piliostigma thonningii* (781.70± 232.9 mg/ kg). The manganese level was high in comparison with other seeds like the Sesemum indicum (10.3 \pm 2.0 mg/kg), Piliostigma thonningii $(1.0 \pm 0.02 \text{ mg/}$ kg), and had a level close to that of Moringa oleifera (17.5 ± 0.4) but lower than what was reported for Cola millenii (100.2 \pm 0.28 mg/kg). The zinc content showed moderate levels when compared to other seeds like Lophira lanceolata ($200 \pm 4.26 \text{ mg/kg}$), and *Cola millenii* ($319.05 \pm 0.35 \text{ mg/kg}$).

The results suggest the seed kernel could be a good source of calcium, magnesium, manganese, iron and zinc, where intakes of 150 - 1000 g, 160 - 850 g, 0.16 - 145 g, 8-32 g and 166 - 786 g would meet the Adequate Intake (AI) and Recommended Dietary Allowance for these minerals respectively (Otten *et al.*, 2006).

Phytochemical content and antioxidant activity

Phytochemical analysis of the seed kernel (Table 3) showed the absence of saponin, oxalates and flavonoids but presence of tannins and phytates in very low amounts which supports report for the fruit pulp as reported by Adepoju (2009). The tannin content reported was lower than other edible seeds in the Nigerian climate such as the Cola millenii (0.127%), Moringa Oleifera (0.3229%), and Bombax glabra (0.12%). The phytate was also lower compared to results submitted for Cola millenii (0.39%) and Bombax glabra (0.054%) (Bello et al., 2008; Kawo et al., 2009). As the presence of phytic acid in foods can cause mineral deficiencies through binding with essential minerals (Bello et al., 2008), the very low amounts submit its use as food without possible decrease in the bioavailability of essential minerals through absorption.

The results of total phenolic contents and antioxidant activity are presented in Table 4. The seed kernel of Spondias mombin exhibited total phenolic content of 573.32 \pm 11.5 mg GAE/100g which is higher than that obtained for the fruit pulp, 367.36 ± 9.53 mg/100g and 260.11 ± 11.89 mg GAE/100g (Tiburski et al., 2011; Olayiwola et al., 2013). In comparison with other seeds, this seed has a higher total phenolic contents than the Artocarpous heterolphyllus seed (212 \pm 0.9 mgGAE/100g), Carica papaya seeds (30.32 ± 6.9) mgGAE/100g), and Canarium odontophyllum Miq. $(400 \pm 40.0 \text{ mgGAE}/100\text{g})$ but inferior to sunflower seed (1601 mgGAE/100g), and buckwheat seed (726 mgGAE/100g) (Velioglu et al., 1998; Prasad et al., 2010; Gupta et al., 2011; Maisarah et al., 2013).

Sample	Tannin (%)	Phytate (%)	Saponin (%)	Oxalate (%)	Flavonoid (%)
Seed kernel	0.06	0.0022	-	-	-
^a Fruit Pulp	0.0024	0.00021	0.00106	0.00188	-

 Table 3 Phytochemical content of Spondias mombin seed kernel and fruit pulp

^a Adepoju (2009). Values are mean \pm standard deviation (n=3)

Seed Kernel Type	Total Phenolic Contents (mg GAE/100g)	Total Antioxidant Capacity (mg AAE/100g)	Free Radical Scavenger (%)
Spondias mombin	573.32 ± 11.50	856.70 ± 5.84	15 ± 0.02
^a Moringa oleifera	-	5620	-
^b Abelmoschus esculentus	-	-	70
^c Artocarpous heterolphyllus	212 ± 0.90	-	45
^d Canarium odontophyllum	400 ± 40.00	-	17 ± 0.30
°Carica papaya	30.32 ± 6.90	-	-
fSunflower	1601	-	-
^g Buckwheat	726	-	-

Table 4 Antioxidant Activity and Phenolic content of Spondias mombin and other seeds kernels

^aKawo *et al.* (2009); ^bAnsari *et al.* (2005); ^cGupta *et al.* (2011); ^dPrasad *et al.* (2010); ^cMaisarah *et al.* (2013); ^{f.g}Velioglu *et al.* (1998). Values are mean \pm standard deviation (n=3)

Results indicate that *Spondias mombin* seed contain phenolic compounds that may provide health benefits and excellent antioxidant dietary sources for reducing risk of chronic diseases (Chang *et al.*, 2007).

The total antioxidant capacity of *Spondias* mombin obtained was $856.7 \pm 5.84 \text{ mg AAE}/100\text{g}$ which was lower than the values obtained for *Moringa Oleifera* seeds reported at 1820 mg AAE/100g and 5620 mg AAE/100g using free and bound phenolic extracts respectively (Singh *et al.*, 2013) but higher than results reported for kiwi and white grape seeds, 739 and 482 mg Trolox equivalent/100g respectively (Duda-Chodak and Tarko 2007). Tiburski *et al.* (2011) obtained 17.47 \pm 3.27 mmol Trolox equivalent/g for *Spondias mombin* L. pulp using the ABTS⁺+assay and classified it as having antioxidant activity above average.

The free scavenging activity of *Spondias mombin* seed against the DPPH radical $(15.10 \pm 0.02\%)$ using methanol extract was lower than that reported for *Abelmoschus esculentus* fruit (70%) and *Artocarpous heterolphyllus* seed (45%) using acetone extract (Ansari *et al.*, 2005; Gupta *et al.*, 2011) but close to that of *Canarium odontophyllum* seed (17 \pm 0.3%) obtained using butanol extract (Prasad *et al.*, 2010).

Conclusion

The Nigerian hog plum (*Spondias mombin*) seed exhibits high nutritional, functional and pharmacological importance. The nutrient composition is higher than its fruit pulp. Data also indicated low concentrations of anti-nutrients, high levels of polyphenols and presence of antioxidant capabilities from the phenolic contents, antioxidant capacity, and free radical scavenging activity measured. The seed kernel of *Spondias mombin* can be a potential source of nutritious food with good anti-anaemic and anti-diabetic function for its high iron and zinc contents.

Acknowledgements

The authors are grateful to the staff and management of The Nigerian Institute of Science and Laboratory Technology (NISLT), Ibadan and the department of Food Technology, University of Ibadan, Nigeria.

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