



Phytochemical Constituents of Flour and Composite Bread from African Palmyra (*Borassus aethiopum*) Fruit from Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CA and MP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OAB reviewed and structured first draft of manuscript and authors CA and CL managed the analyses of the study and performed final review input and proofreading of manuscript. Authors MP and OAB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To explore the use of *Borassus aethiopum* fruit as composite flour and bread and determine the phytochemical composition in each product.

Place and Duration: Department of Biochemistry, and Department of Food science food and sensory evaluation laboratory, between September, 2016 and April, 2017.

Study Design: An experimental study.

Methods: The African palmyra fruits were obtained from Sekyere Odumase and processed into *Borassus* flour and composite bread. Phytochemicals constituents were determined in both products using aqueous and methanol extracts.

Results: The results of both aqueous and methanol extraction for the phytochemical testing indicated that the raw *B. aethiopum* powder (RBAP) contained flavonoids, saponins, phenols, cardiac glycosides, alkaloids, triterpenes, steroids and sterols. *Borassus* bread contained

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flavonoids, alkaloid, triterpenes, steroids and sterols in both extracts, whilst cardiac glycosides, saponins and phenols maintained their strong presence in both extracts of composite Borassus bread. Tannins were absent in both extracts of *B. aethiopum* powder and bread composite. Also, the total phenol content in the composite bread was lower compared with the Borassus flour ($P=0.000$). The total antioxidant capacity in the Borassus flour ($EC_{50}=2.1\pm 0.24$ mg/mL) was significantly higher than the composite bread ($EC_{50}=2.24\pm 0.4$ mg/mL) ($P=0.000$). However, both products had less antioxidants than standard ascorbic acid ($EC_{50}=0.12\pm 0.2$ mg/mL).

Conclusion: Both *Borassus* flour and composite bread had appreciable phytochemicals present, with some antioxidant capacity which could beneficially help in the management of people with chronic diseases such as diabetes and cardiovascular diseases.

Keywords: Phytochemicals; *Borassus aethiopum*; total phenols; antioxidant; African palmyra.

ABBREVIATIONS

RBAP/F : Raw *Borassus aethiopum* Powder/
Flour

PBAB : Processed *Borassus aethiopum* Bread

DPPH : 2, 2-diphenyl-1-picrylhydrazyl radical

1. INTRODUCTION

Borassus aethiopum Mart is a tropical plant of the family *Arecaceae* [1] grown widely across countries within sub-Saharan Africa. Across the African continent, the shape of the leaf, resembling the palm of the hand, has assumed several names including "African Palmyra palm" and "African fan palm" [1]. In Ghana, like other African countries, it is used as food and for other purposes, and depending on the geographical location, notable local names of African Palmyra palm include 'oman kube' or "besia kube" by the Ashanti's, found in the 'Abrimasu' forest reserve of Mampong, Kobreti, Kwaseakan, Teacherkrom, Adome and Aframso [2]. In the Volta region of Ghana, it is called "agoteku" and grown in areas; Adaklu, North Tongue, Kpetoe-Ziope and Akatsi North [2]. It is one of the less known and underutilized tropical fruits, with potential health benefits [2].

The unripe fruits are greenish, turning dull orange-brown when ripe, and approximately weigh 1-1.5 kilogram. The shape of the fruit, normally ovoid or triangular, is dependent on the number of seeds present (usually 2-3). The pericarp is tough, and has a woody endocarp, with a yellowish, slightly oily pulp containing the seed. The length of the *Borassus* seed is 10cm, and weighs 100g, having *Borassus* fruit a short viability [1].

As a functional plant, various parts of the *Borassus* plant can be used for nutritional and medicinal purposes [2]. As medicinal plant, the wood of the *Borassus* plant is resistant to

termites and fungi [3] the root in treating fungal and viral infections including measles [4] asthma and impetigo [3], anti-pyretic ability [5] some sexually transmitted infections [6] and has anti-helminthic [8,9] function. The nutritious part of the plant, the fruit; when eaten raw or cooked contains nutrients; carbohydrate, provitamin A and vitamin C, and protein [10] and has been demonstrated to be antidiabetic in animal models [11].

Also, the pulp of the fruit is known to contain phytochemicals; flavonoids, alkaloids, triterpenes, cardiac glycosides, sterols and steroids [10,12,13,11] which exhibits some antioxidants [13, 14], antiplasmodial properties [12] and anti-inflammatory [14] properties. In Ghana, the fruit is seasonally available, abundant, and inexpensive, however, its use is limited due to poor knowledge on its nutritive and medicinal values [1]. Additionally, the *Borassus aethiopum* fruit is underutilized in Ghana due to its bitter taste, the tough and hard nature of the pericarp and seed, which makes it unpleasant waste for food. This has caused seasonal wastage of the edible, nutritious *Borassus aethiopum* fruits in Ghana [1]. Issaka et al. [11] reported some phytochemicals such as tannins, glycoside, and flavonoids to be present in the *Borassus aethiopum* fruit hence the processing of the fruit pulp into food products could provide these phytochemicals, with health benefits. Research into the phytonutrients profile of the extracted fruit pulp had been done [11] but the phytochemicals constituents of processed and edible fruit pulp such as making bread and powder from the *Borassus aethiopum* fruit for consumption had not been exploited. Therefore, this study sought to explore the phytochemical constituents of the *Borassus* powder and composite bread of *Borassus aethiopum* fruit from Ghana.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fully ripened and matured Palmyra palm fruits were obtained from Sekyere Odumase in the Ashanti region of Ghana.

2.2 Powdered Palmyra Fruit

Ripe palmyra fruits were washed thoroughly, the outer thick skin (exocarp) peeled off and fibrous part of fruit pulp (mesocarp) separated from the seed. The fleshy layer was chopped into small pieces and spread in clean trays and dried in a conventional solar dryer. The drying period was dependent on the sun's heat. Dried samples were then milled into powder (Raw *Borassus aethiopum* Powder, RBAP) using a hammer mill, containing sieve with 4.0 mm particle size sieves, and finally stored in black polythene bags at temperatures below 4°C for further use.

2.3 Bread Development

Modifications were made to Jamie Oliver's basic bread recipe [15] for the *Borassus* composite bread. The RBAP was used as composite flour for the dough. Two different flour (white and wheat) were used in the ratios of 2:1, 5:1, 10:1 and 20:1 flour to RBAP. Other ingredients, margarine, sugar, salt, instant yeast, diluted milk, nutmeg, egg and some drops of essences were used. Ingredients were carefully weighed, mixed to form a dough, kneaded, and dough weighed to give accurate weight per serving. The dough was baked in a preheated oven between temperatures of 250-300°C for 25-40 minutes. Sensory analysis was performed data of which is not included in the current study [15].

2.4 Aqueous Extract

Aqueous extraction was done using the protocol described by Shanmugum et al. [16] with few modifications to the RBAP and bread (Processed *Borassus aethiopum* Bread, PBAB) separately. Twenty grams each of dried sample was added to 200 ml of water, boiled for 5 mins and allowed to cool, filtered through Whatman no. 1 filter paper and volume adjusted to 20 g/200 mL. The extract was then used for the various qualitative tests [16].

2.4.1 Methanol extraction

Alcoholic extraction method according to Cowan (17) was used. Twenty grams of dried samples

were soaked in 200 ml of methanol to obtain 20 g/200 mL, for extended 24 hours. The mixture was then filtered through Whatman no. 1 filter paper and washed with 50 ml of methanol and the residue dried. The extracts were used directly for the qualitative phytochemical screening.

2.5 Phytochemical Analyses

Phytochemicals present in the aqueous and methanol extracts of RBAP and PBAB were determined using standard methods. The modified Prussian blue (for Tannins), Salkowski (for terpenoids), Liebermann Burchard, Shinoda (for flavonoids), Dragendoff's (for alkaloids), Keller-Killani test (for glycosides) and froth test (for saponins) described by Shanmugam et al. [16] were used in the phytochemical testing of both aqueous and methanol extraction of RBAP and PBAB.

2.5.1 Determination of total phenolic content

Stock solution of the extract was prepared by diluting 10 mg of each of the samples in 1 ml water. A stock solution of 5 mg/mL of standard (gallic acid) was prepared by dissolving 50 mg of it in 1 mL absolute ethanol. This was then diluted in 9 mL distilled water to obtain the 5 mg/mL stock solution. A fivefold serial dilution on the gallic acid standard obtained six different concentrations 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/mL. A water blank was also prepared. A five-fold serial dilution of the 20 mg/ml extract obtained three different concentrations (10, 5, 2.5 mg/mL). Methanol only was also prepared as blank. Ten µL of the sample and gallic acid dilutions were aliquoted into a 2.0 mL Eppendorf tubes. Aliquots of 790 µL of distilled water were then added, followed by the addition of 50 µL of Folin-Ciocalteu reagent. The tubes containing mixture were vortexed for five seconds. The tubes were incubated in darkness at room temperature for eight minutes. A volume of 150 µL of 7% sodium carbonate solution was added to each tube, and vortexed for five seconds, and further incubated in darkness at room temperature for two hours. After the incubation, 200 µL of the extract and gallic acid standard dilutions was aliquoted into a 96-well plate (in triplicate) and absorbance read at 750 nm using microplate spectrophotometer (Synergy H1). A graph of absorbance against concentration was plotted for the gallic acid standard. The concentration of total phenolic in the extract was determined using the gallic acid standard plot [16].

2.5.2 Free radical (DPPH) scavenging ability

Stock solution of the extract was prepared by dissolving 10 mg of the dried bread in 1 ml of water. Also, stock solutions of 10 mM of standard (ascorbic acid) and 0.5 mM of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were prepared by dissolving 0.176 mg of ascorbic acid, and 3 mg of DPPH in 1mL of water, and 15 mL absolute methanol respectively. The solutions were vortexed to complete dissolution. The DPPH solution was immediately kept in the dark. Serial dilution on the bread extract was done to obtain concentration ranges from 0.156–10 mg/ml. Hundred microliters of each concentration of the test sample was transferred into a 96-well plate. This was followed by the addition of 100 μ L of 0.5 mM DPPH. For positive control, ascorbic acid was used at a concentration range of 0.156–10 mM in distilled water. Distilled water was used as blanks. Triplicate experiments were performed. The plates were covered with aluminum foil, shaken gently and kept in the dark for 20 minutes, after which the absorbance was read on a Synergy H1 reader at 517 nm. Percentage scavenging activity was determined by;

$$\% \text{ scavenging} = \left[\frac{\text{Absorbance of blank (OD0)} - \text{Absorbance of test (OD1)}}{\text{Absorbance of blank (OD0)}} \right] \times 100$$

Absorbance of blank (OD0)

The effective concentration at 50% (EC_{50}) values, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, were determined by nonlinear regression analysis [16].

3. RESULTS AND DISCUSSION

The free radical scavenging activity of both flour and bread samples is presented in Table 1. The role of antioxidants in health is its ability to interact and scavenge oxidative free radicals. In summary, with the DPPH method, antioxidants react with stable free radical, α , α -diphenyl- β -picrylhydrazyl, and converted to α , α -diphenyl- β -picrylhydrazine, showing discolouration. In the present study, the flour and the bread samples decolourised DPPH, showing its free radical scavenging potentials. The Borassus flour ($EC_{50} = 2.1 \pm 0.2$ mg/ml) had higher antioxidant capacity compared to Borassus composite bread ($EC_{50} = 2.2 \pm 0.4$ mg/ml). The low antioxidant level

in the Borassus composite bread may be attributed to the heating process during baking. However, the antioxidant content of Borassus composite bread ($EC_{50} = 2.2 \pm 0.4$ mg/ml) and Borassus flour ($EC_{50} = 2.1 \pm 0.2$ mg/ml) was significantly lower than the standard ascorbic acid ($EC_{50} = 0.124 \pm 0.02$ mg/ml; $p = 0.000$). The result indicates that the Borassus fruit pulp contains some antioxidant levels, which can prevent oxidative damage. Although, the amount of antioxidant capacity in the Borassus composite bread and Borassus flour was lower than ascorbic acid, the amount present could be very beneficial in improving endogenous total antioxidant capacity when consumed. The addition of *Borassus aethiopicum* fruit pulp into bread flour could provide these antioxidants, with a lot of health benefits, such as protecting against oxidative disease including diabetes and cardiovascular diseases. Hence, advocacy on the consumption of the Borassus fruit can serve as inexpensive food with health benefits for the population. The *Borassus aethiopicum* fruit pulp can be use as flour in cookery and as composite flour for bread and provide same nutritive values.

Additionally, the result showed the total phenolic content of the two samples the Borassus composite bread (0.72 ± 0.11 units) had lower total phenolic content than in the flour (1.14 ± 0.55 , in Table 1). According to Amoateng et al. [14] and Wood et al. [18] a positive relationship existed between antioxidant activity potential and phenolic compounds in a substance. This means increasing total phenolic content could increase the antioxidant capacity. Therefore, the result showed that the Borassus flour contains higher levels of antioxidant capacity compared to Borassus composite bread. The low total phenolic content in Borassus composite bread could be attributed to some protein ingredients used in the bread making. Proteins are known to bind to phenolic compounds, thus inhibits its availability and release in food [18]. It was also recorded that, peels contain more phenols than the pulp of some coloured fruits. Other study also showed that peeling of the Borassus fruit pulp gets rid of microorganism, which breaks the natural barriers between the fruit content and outer part of the fruit, thus, reducing their total phenolic content [19]. The bread that contained a ratio of 10:1 white flour to Borassus flour was the preferred choice after sensory analysis (data not presented in the current study).

Table 1. Antioxidant and total phenolic content of borassus powder and composite bread compared with standards (ascorbic acid and gallic acid)

Sample	Mean EC ₅₀ (mg/mL)	P value
Ascorbic acid	0.12 ± 0.02	0.000*
<i>Borassus aethiopum</i> powder	2.11 ± 0.24	
<i>Borassus aethiopum</i> bread	2.24 ± 0.42	0.000*
Sample	Total phenolic content	
Gallic acid	4.94 ± 0.01	0.007*
<i>Borassus aethiopum</i> powder	1.14 ± 0.55	
<i>Borassus aethiopum</i> bread	0.72 ± 0.11	0.000*

*P-value is significant at $p \leq 0.05$

Table 2. Phytochemical screening of aqueous and methanol extract of RBAP and PBAB

Parameters	RBAP		PBAB	
	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract
Flavonoids	++	++	-	+
Saponins	++	+	++	+
Glycosides	++	++	++	++
Alkaloids	-	+	-	+
Tannins	-	-	-	-
Steroids and Sterols	-	++	-	+
Triterpenes	+	+	+	+
Phenolics	++	++	++	++

RBAP- Raw *Borassus aethiopum* Powder, PBAB- Processed *Borassus aethiopum* Bread, ++ indicate strong presence, + indicates weak presence, - = absence

The aqueous and methanol extracts of RBAP and PBAB were screened for the presence of phytochemicals. The results (Table 2) indicated that the RBAP and PBAB had a significant presence of phenols and cardiac glycosides in both extracts, whereas alkaloids, flavonoids, saponins were weakly present. Triterpenes were fairly present in both extracts of the RBAP and PBAB. Alkaloids, flavonoids, and sterols and sterols were sparingly present in either aqueous extracts or methanol extract of the RBAP and PBAB. An earlier study by Issaka et al. [11] found the presence of tannins, saponins, glycosides, alkaloids and triterpenoids in the fruit extract, which was consistent with findings of our study. This suggests that RBAP and PBAB retained the phytonutrient compounds, even after processing into edible food, and thus can provide such health benefits when consumed.

Studies by Small [2] and Vijayakumari et al. [20] also identified phytochemicals including phenol, saponins, terpenes, sterols and steroids, cardiac glycosides and flavonoids in the *Borassus aethiopum* fruit pulp. These phytochemicals observed in the *B. aethiopum* fruit pulp have

important health benefits in the body. Flavonoids attenuate the development of atherosclerosis formation, through preventing oxidative damage by reactive oxygen species (ROS) [21,22]. Also, the cardiac glycoside is known to act directly on the Na⁺/K⁺-ATPase pump, preventing energy from reaching the pump or interfere with its carrier mechanisms [23,24]. Cardiac glycosides additionally have a positive inotropic effect, necessitating its use as an anti-arrhythmic agent on the heart muscles in cases of cardiac insufficiency [23,24]. Saponins possess membranolytic properties that assist in the formation of micelles with bile salt in the body. This properties of saponins inhibit absorption of lipids (cholesterol) and facilitate its excretion via the intestinal tract [25]. These phytochemicals present in both products from the *B. aethiopum* fruit pulp may provide antioxidant, anti-inflammatory and anti-atherogenic effects [13,1]. As inexpensive and abundant as the *B. aethiopum* fruit is, there is the need for industrial processing into other products which could provide health benefits in the management of people with chronic diseases such as diabetes and cardiovascular diseases.

4. CONCLUSION

The processing of the *B. aethiopum* fruit pulp into flour and composite bread contained the presence of phenol, saponins, terpenes, sterols and steroids, cardiac glycosides and flavonoids in appreciable amounts. The *Borassus aethiopum* fruit pulp can be utilized as a food or incorporate in food products, to provide us with these phytochemicals with health benefits necessary to help prevent/manage diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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