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Nutritional and Phytochemical Analysis of Ripe and Unripe *Roystonea regia* Fruit Pericarp

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Abstract

This study investigated the potential of Roystonea regia fruit pericarp for use as food and medicine. This was done by comparatively assessing the mineral, proximate and phytochemical compositions of ripe and unripe fruit pericarps. Results of phytochemical screening of the pericarp ethanol extract showed that reducing sugar, tannin, saponin, cardiac glycoside, alkaloid, phenolic and flavonoid were present in both ripe and unripe fruit pericarps while anthocyanosides and anthraquinones were not detected in both. Results of the proximate analysis showed that the ripe pericarp contained more moisture (7.58 %), crude protein (8.07 %), ash (11.14 %), fat (9.80 %) and carbohydrate (39.86 %) while the unripe pericarp contained more dry matter (92.90 %) and crude fibre (30.56 %). However, results of the mineral analysis showed that Ca, Mg, K, Na, Mn, Fe, Zn and Ni were higher in the unripe pericarp while both pericarps had the same concentration of P (119.95± mg/100g). Co, Cd and Pb were not detected in both samples while Ni (0.73 mg/ 100g) was found only in the unripe pericarp. Relative to other minerals, K was highest in both ripe (1600.24 mg/100g) and unripe pericarp (1804.42 mg/100g). Based on the presence of pharmaceutically important phytochemicals, essential minerals and proximate values, this study concluded that both ripe and unripe R. regia pericarps have significant nutritional and medicinal potentials which could be exploited domestically and industrially.

Keywords: *Roystonea regia, phytochemical, mineral, proximate, fruit pericarp*

Introduction

Roystonea regia is an ornamental palm tree popularly known as the Cuban royal palm, Florida royal palm, or simply the royal palm. It is native to Southern Florida, Mexico and parts of Central America and the Northern Caribbean (Royal Botanic Gardens, 2009). Although it is sometimes called *Roystonea elata*, the conserved name *R. regia* is now the correct name for the species (Döring M, 2015). Its flowers are visited by birds and bats and it serves as a roosting site and food source for a variety of animals. *Roystonea regia* is the national tree of Cuba (Zona-Scot, 1996) and has a religious role both in Santería and Christianity, where it is used in Palm Sunday observances. The plant reaches a height of 20–30 metres tall and stem diameter of about 47 centimetres. The trunk is stout, very smooth and grey-white in colour with a characteristic bulge below a distinctive green crownshaft. Trees have about 15 leaves which can be up to 4 m long. The flowers are white with pinkish anthers. The fruits are spheroid to ellipsoid in shape, 8.9–15 millimeters long and 7–10.9 mm wide. They are green when immature, turning red and eventually purplish-black as they mature (Carbajal *et al.,* 2009). The fruit is eaten by birds and bats (which disperse the seeds) and fed to livestock.

From our literature search, as at the time of putting this write up together, not much has been reported on the plant especially the fruits. However, an extract from *R. regia* fruit known as D-004 has been reported to reduce benign prostate hyperplasia (BPH) in rodents. D-004 is a mixture of fatty acids and is being studied as a potential alternative to finasteride for the treatment of BPH (Dransfield *et al.,* 2008). Also, the root was reportedly used as diuretic and therefore as a remedy for diabetes (Austin and Daniel, 2004). It is used in Cuba for timber, thatch and hog feed while it is leaf is prepared as a decoction for medicinal and veterinary purposes for the treatment of nervous system and mental health related issue and also as an aid to treat the digestive system in Peru (Carbajal *et al.,* 2009).

The study was carried out to investigate the proximate, mineral and phytochemical compositions of the pericarps of ripe and unripe *Roystonea regia* fruit. This is in order to ascertain its potential usefulness for food and medicine.

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Material and method

Collection and Preparation of sample

Fresh, mature and healthy fruits (ripe and unripe) were collected from the same *R. regia* tree within Babcock University, Ilisan-Remo, Ogun State, Nigeria. The fruit samples were identified by Professor Edward Esan, a Professor of Botany in the Department of Basic Sciences, Faculty of Science and Technology, Babcock University. The fruits were separated from the stalk, washed thoroughly and air-dried at room temperature. The pericarp of the air-dried fruits were manually removed and pulverized with the use of a laboratory blender (Lexus MG-2053 Optima). The pulverized samples were analyzed for proximate, mineral and phytochemical compositions.

Extraction of sample

Ethanol extracts of the samples were prepared by cold maceration using the method described by Usunobun *et al*. (2015). 100 g of each of the pulverized samples was macerated separately in 1 L of absolute ethanol at room temperature for 48 hrs. The resulting mixture was filtered with a Whatmann filter paper No. 42 and then through cotton wool. The extract was thereafter concentrated under reduced pressure with the use a rotary evaporator (Eyela N-1001) at 40 ^{o}C to one-tenth its original volume and then finally freeze-dried. The freeze-dried ethanol extract was then stored at 4 ºC and subsequently used for phytochemical screening.

Phytochemical screening

Phytochemical screening of the ethanol extract was carried out by using standard methods described by Sofowora, 1993; Trease and Evans, 1989 and Ayoola *et al.,* 2008.

Test for Saponins

0.5 g of extract was added to 5 mL of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. It was thereafter observed for the formation of an emulsion.

Test for Tannins

0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride were added and the solution observed for brownish green or a blue-black coloration.

Test for Reducing Sugar (Fehling's Test)

0.5 g of the extract was dissolved in 5 mL distilled water and filtered. The filtrate was hydrolyzed with dilute HCl, neutralized with NaOH solution and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Test for Anthraquinones

0.5 g of the extract was boiled with 10 mL of H_2SO_4 and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipette into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for color changes.

Test for Cardiac Glycosides (Keller-Killiani Test)

To 0.5 g of extract dissolved in 5 mL water was added 2 mL of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for Flavonoids

5 mL of dilute ammonia was added to 2 mL of the extract and shaken. 1 mL of concentrated Sulphuric acid was then added. A yellow coloration indicated the presence of flavonoids.

Test for Alkaloids

0.1 g of the extract was dissolved in 5 mL dilute HCl and filtered. The filtrate was treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids.

Test for Phenol

2 mL of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

Proximate analysis

Proximate analysis of the pulverized sample; dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre was carried out by the standard method of the Association of Official Analytical Chemists (AOAC, 2000). Total Carbohydrate content was obtained based on the net difference between the sum of other parameters and the total percentage composition i.e. 100- (% moisture + % crude protein + % crude fat + % crude fibre + % ash content).

Mineral analysis

Modified method of AOAC, (1990) was used to determine the mineral content of the sample. 1.0 g of pulverized sample was measured into a pre-weighed crucible and incinerated in a muffle furnace at 550 °C for 6 hrs. The resulting ash was dissolved in 50 ml of 10 % HNO_3 solution. The solution was reduced to 25 mL by heating on a hot plate and immediately filtered while hot with a Whatman No. 1 filter paper. The concentrations of Ca, P, Cd, Zn, Mg, Mn, K, Na, Pb, Co, Ni and Fe were determined in the ash solution by using Atomic Absorption Spectrophotometer (Buck Scientific Model 2010 VGP) except P which was determined with a UV-Spectrophotometer (LaboMed SPECTRO SC). Reagent blank was concomitantly prepared by boiling 50 mL of 10 % HNO_3 on a hot plate to reduce its volume to 25 mL. It was filtered while hot and analyzed in the same manner as the sample. Results are means of three replicates.

Results and discussion

Table 1: Phytochemical Screening of ripe and unripe fruit pericarp of *R. regia*

Phytochemicals	Ripe pericarp	Unripe pericarp
Reducing Sugar	+++	+++
Tannins	+++	+++
Saponins	++	+
Cardiac glycosides	++	+++
Alkaloids	+++	+++
Phenols	+++	++
Flavonoids	+++	++
Anthocyanosides	ND	ND
Anthraquinones	ND	ND

+++= highly present, ++= moderately present, += slightly present, ND= not detected

Phytochemicals are natural bioactive compounds present in plants that work with nutrients and dietary fiber for protection from diseases (Oteng-Gang and Mbachu, 1990). Table 1 shows the results of the phytochemical screening of both the ripe and unripe pericarps of *R. regia* fruit. The results showed that reducing sugar, tannin and alkaloids were highly present in both ripe and unripe samples. Phenols and flavonoids were moderately present in the unripe but highly present in the ripe fruit pericarp. Saponin was slightly and moderately present in unripe and ripe sample respectively. Cardiac glycoside was highly present in the unripe sample and moderately in the ripe. Meanwhile, anthocyanoside and anthraquinones were not detected in both samples. Medicinal or pharmacological activities of plant extracts have been attributed to the presence of some of these phytochemicals. This suggests that the pericarp of *R. regia* fruit would possess important pharmacological potentials. For example, anti-bacterial activity of plant extracts has been attributed to the presence of alkaloids (Idowu *et al.,* 2003). Also, several alkaloid containing medicinal plants are reported to have been used as pain relievers, recreational stimulants or even in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich *et al*., 2005; Gurib-Fakin, 2005). Tannin-containing plants are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001). Tannins are also known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu and Emineke, 2006; Li *et al*., 2003; Adegboye *et al.*, 2008). Flavonoids and phenol are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al*., 2013; Pourmorad *et al*., 2006; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995). However the presence of flavonoids and phenols is in agreement with the work of Kumar *et al* (2013) who attributed the antioxidant properties of *R. regia* to the presence of these phytochemicals. Cardiac glycosides are important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure (Yukari *et al*., 1995). This phytochemical has medicinal value in cardiac arrest prevention. When consumed, it elevates the intracellular calcium concentration, thereby increasing cardiac output through an increase in the force of contraction of the heart (Shamaki *et al.,* 2012).

Table 2: Proximate composition of ripe and unripe fruit pericarp of *R. regia*

Proximate Composition (%)	Ripe pericarp	Unripe pericarp
Dry Matter	92.42±0.12	92.90±0.11
Moisture	7.58±0.22	7.10±0.14
Crude Protein	8.07±0.04	6.93±0.10
Crude Fibre	23.55±0.12	30.56±0.21
Ash	11.14±0.11	10.46±0.12
Crude Fat	9.80±0.02	7.40±0.03
Carbohydrate	39.86±0.01	37.55±0.02

Data are expressed as mean ± standard error of three replicates

Results of the proximate composition of ripe and unripe fruits pericarp of *R. regiȧs* are presented in Table 2. The results showed that in comparison to other parameters, carbohydrate content was highest while protein content was lowest in both samples. The relatively low moisture content observed in both ripe (7.58 %) and unripe (7.10 %) pericarps could enhance inhibition of microorganism growth and elongate storage life of the samples (Danlami *et al.,* 2012). The crude fibre content of the unripe pericarp (30.56 %) was higher than the ripe (23.55 %). Fibre works as a good cleanser of the digestive system by removing carcinogenic substances from the body and preventing cholesterol absorption. Fibre also prevents

excess consumption of food by reducing digestion of carbohydrate and delays its absorption hereby promotes weight loss. The significant amount of fibre in both ripe and unripe *R. regia* pericarps indicates that they could help in protecting the digestive system and aids weight loss (Usunobun *et al*., 2105, Mensah *et al.*, 2008). The pericarp of *R. regia* fruit contained 8.07 % and 6.93 % crude protein in ripe and unripe respectively. Protein generally is crucial for animal growth and it increases milk production. Its substantial amount is of great significance to human health especially breastfeeding mothers and growing children. Plant proteins are a main source of food nutrient particularly for the poor populace in developing countries like Nigeria. Protein is an essential macromolecules and it is an alternate energy source when other energy sources are in short supply. Food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes and necessary elements for the manufacture of DNA (Okerulu and Onyema, 2015; Bailey, 2008). *R. regia* fruit pericarp can hence be considered a good source of protein due to the presence of substantial amount of crude protein. The ash content of 11.14 % and 10.46 % were obtained for both ripe and unripe samples respectively and this indicates that the pericarps of *R. regia* are rich in mineral elements. The crude fat content was found to be 9.80 % and 7.40 % for both ripe and unripe respectively. Dietary fat are reported to increase the tastiness of food by absorbing and retaining flavours (Antia *et al*., 2006). The carbohydrate contents of the samples were 39.86 % and 37.55 % for both ripe and unripe respectively. Carbohydrates are largely categorized as polysaccharides or indigestible carbohydrates are known to hinder colonization of pathogenic microbial flora in the intestines, therefore, they assist removal of these pathogens from the gut system accompanied by improved immunity (Guo *et al*., 2003). Carbohydrates are crucial for the preservation of life in both plants and animals. Carbohydrates in plants are one of the three main energy sources in food with protein and fat inclusive.

Table 3: Mineral composition of ripe and unripe fruit pericarp of *R. regia*

S/N	Mineral	Ripe pericarp (mg/100g)	Unripe pericarp(mg/100g)
1	Calcium	370.46±0.23	608.24±0.03
2	Magnesium	99.57±0.22	117.70±0.05
3	Potassium	1600.24±0.22	1804.42±0.03
4	Sodium	111.36±0.15	116.08±0.11
5	Manganese	1.44±0.11	2.78±0.12
6	Iron	6.04±0.02	9.10±0.03
8	Phosphorus	119.95±0.32	119.95±0.31
9	Zinc	1.264±0.10	1.622±0.10
10	Cobalt	ND	ND
11	Cadmium	ND	ND
12	Lead	ND	ND
13	Nickel	ND	0.734±

Data are expressed as mean ± standard error of three replicates

Minerals play a key role in maintaining proper function and good health in the human body. Insufficient consumption of minerals in the diet is often linked to an increased susceptibility to infectious diseases due to the weakening of the immune system (Sajib *et al*., 2014). The mineral analysis of *R. regia* fruit pericarp (Table 3) showed the presence of eight minerals in the ripe and nine in the unripe. The results showed that the unripe pericarp had higher concentrations of all the minerals (Ca, Mg, K, Na, Mn, Fe, P, Zn and Ni) detected except phosphorus which occurred at equal concentration of 119.95 mg/100g in both. This implied that the concentration of phosphorus was not altered by ripening; that is, equal amount can be obtained in either ripe or unripe pericarp of the fruit. Meanwhile, nickel which showed a concentration of 0.734 mg/100g in the unripe pericarp was not detected in the ripe. Also, Co, Cd and Pb were not detected in both pericarps. The minerals detected in *R. regia* fruit pericarp have been reported to possess therapeutic importance required for normal growth, muscular activity, skeletal muscle development and other body functions. Calcium is essential for bone formation and its maintenance, teeth, muscles and blood coagulation whose shortage may leads to rickets, curvature of the spine and pelvic deformities (Okerulu and Onyema, 2015). The presence of magnesium in these pericarps suggests that they could be helpful in preventing cardiomyopathy, impaired spermatogenesis and bleeding disorders (Chaturvedi, 2004). Potassium is the third most abundant mineral in the human body and it is an important element in improving human health. It functions include relief from stroke, blood pressure, heart and kidney disorders, anxiety and stress (Poirier, 1984; Sacks *et al.,* 1998). The presence of high content of potassium therefore makes these samples good for medicinal purposes. Sodium is a vital mineral that is required for humans to maintain the balance of the physical fluids system. It is also necessary for proper functioning nerve and muscle. Although sodium is a needed nutrient in any balanced diet, it can also cause stomach cancer and hypertension if not cautiously taken (W.H.O, 2008). Manganese is part of enzyme involved in urea formation, pyruvate metabolism and the galactotransferase of connective tissue biosynthesis (Chandra, 1990) and its deficiency has been linked to retardation of growth, reproduction and skeletal anomalies. Iron is essential in the formation of hemoglobin and myoglobin, which function in oxygen-transport. Phosphorus is an important constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids. It acts as buffer to maintain the acid-base balance of cellular fluid because of their ability to combine with hydrogen ion (Elinge *et al*., 2012). Zinc is reportedly important for proper functioning of the reproductive system (Hambidge, 2006).

Conclusion

From the findings of this study, both ripe and unripe pericarps of *Roystonea regia* showed good potential for

food and medicine. However, further studies into the quantification of chemical composition and pharmacological activities of the pericarp are recommended.

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