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QUALITATIVE AND QUANTITATIVE EVALUATION OF THE PHYTOCHEMICALS OF *RAPHIA HOOKERI* AND *RAPHIA FARINIFERA* FRUITS

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Abstract
The shell, pulp and seed of Raphia hookeri and Raphia farinifera fruits obtained from Kwara and Enugu States, Nigeria were investigated. Four solvents (n-hexane, ethyl acetate, methanol and water) were used for extraction prior to phytochemical screening. The results of phytochemical screening revealed that every part of these two fruits species contained phytochemicals, but alkaloids and anthocyanins were not detected in any of the extracts. Terpenoids and cardiac glycosides were present in the methanolic, ethyl acetate and n-hexane extracts of the seed, pulp and shell of the two species, but they were not detected in the aqueous extracts. Saponins were detected mainly in the aqueous and methanolic extracts of the two species but their presence was not significant in other extracts. Quantitatively, tannins content in the pulp of Raphia farinifera was higher than that in Raphia hookeri pulp. Flavonoid was not present in the seed and pulp of either of the two fruits species. The study has shown that Raphia hookeri and Raphia farinifera fruits contain phytochemicals which can be biologically and physiologically active and could have positive medicinal properties.

Keywords*: phytochemicals, qualitative, quantitative, Raphia farinifera, Raphia hookeri, shell, pulp, seed*

Introduction

Raphia is a type of palm which grows in swampy and semi-swampy areas of the equatorial rain forest or derived savannas. About 20 species are known (Ugwu & Igboeli, 2009). Raphia palm is a solid straight monocotyledonous plant belonging to the family of Palmaceae, with trunk covered with attractive unusual coils, and occasionally producing suckers (Hutchinson & Dalziel, 1963).

The Raphia palm is among the eleven indigenous genera of palms found in Nigeria which include *Borassus, Elaeis, Hyphaene, Phoenix, Raphia, Ancistrophyllum, Calamus, Eremospatha, Oncocalamus, Podococcus* and *Sclerosperma.* The detailed morphology, distribution, products and uses of the different species of palms mentioned above have been discussed previously (McCurrach, 1965; Rusesl & Tulley, 1965; Corner, 1966; Hartley, 1967; Otedoh, 1972, 1975, 1976).

Scientific reports and investigations on Raphia palms have shown that its origin is traceable to West Africa, particularly along the swampy area of the tropical forest (Otedoh, 1976; Moore, 1973; Ndon, 2003). Just like *Elaeis guinensis* (oil palm), every part of Raphia palm tree is useful economically. The mesocarp of the ripe Raphia fruit yields edible oil (Otedoh, 1976), Raphia palm wine is a source of beverage and nutrients (Haynes & McLaughlin, 2000), and the palm tree has demonstrated good building construction properties, especially for roofing and ceiling; it has the ability to conduct low heat into the interior space of a building (Akpabio *et al*, 2001), The leaves of raphia palms serve as roofing material and as a source of fiber (from young leaves) used in the fabrication of bags, wallets, shoes, cases, decorations and several works of art (Mphoweh, 2005).

In recent years, it has become clear that phytochemicals have roles in the protection of human health. These compounds are known as secondary plant metabolites and they have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Steinmetz & Potter, 1996, Altiok, 2010). There are more than thousand known and many unknown phytochemicals. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases (Altiok, 2010; Liu, 2004).

Not much work has been done or reported on the phytochemical constituents of *Raphia hookeri* and *Raphia farinifera* fruit, although the fruits have been reported to be cooked and eaten and folklore has it that the cooked plant has some medicinal properties. In this work therefore, the phytochemicals present in the shell, pulp and seed of *Raphia hookeri* and *Raphia farinifera* will be investigated in order to have information about their medicinal importance.

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Materials and Methods

Sampling sites

Raphia hookeri fruit was obtained from Mawokpan village in Edu Local Government Area of Kwara State, Nigeria; while *Raphia farinifera* fruit was obtained from Agbani Nkanu West Local Government Area, Enugu State, Nigeria.

Sampling methods and samples preparation

The fruits (*Raphia hookeri* and *Raphia farinifera* fruits) were collected from the Raphia palm trees using an axe and transferred to the laboratory in different polyethylene bags. The two samples were treated separately, spoilt fruits were separated from the good ones, and the good samples were thoroughly washed with clean water and dried before separating the three parts of the fruit (i.e shell, pulp and the seed) to different vessels. The samples were then air dried at room temperature.

Qualitative phytochemical screening

Samples were air-dried, pulverized and passed through a sieve (about 0.5 mm pore size) to obtain a fine dry powder. Extraction was carried out by maceration using the following solvents separately, n- hexane, ethyl acetate, methanol, and water. 15 g of each sample was separately extracted in 150 ml solvents for 24 hours at room temperature. The extracts were then filtered using whatman filter paper No 42 (125 mm). The filtrates collected were concentrated using rotary evaporator except water extracts which were concentrated on the hot plate and the following tests were carried out. Standard procedures (Trease & Evans, 1989; Sofowora, 1993; Ayoola *et al*, 2008; Savithramma *et al*, 2011) were used in the qualitative determination of the phytochemicals.

Quantitative determination of anti nutrients and phytochemicals

Determination of tannins

5 g of each of the ground sample was weighed into a conical flask and 100ml 2M HCl was added. The content was boiled on a water bath for 30minutes. The extract was cooled and filtered using Whatman No.1 filter paper. The filtrate was taken up twice in 40ml each of diethylether. The ether extract was heated to dryness and weighed (Okwu & Iroabuchi, 2004).

Determination of saponins

5 g of each sample was weighed and dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The sample mixture was filtered by using Whatman No.1 filter paper and the residue was re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight and the saponin content was calculated (Obadoni & Ochuko, 2001).

Determination of flavonoids

10 g of each sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125 mm). The filtrate was then transferred into a 200ml beaker and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated (Boham & Kocipai, 1994).

Determination of total phenolics

A fat free sample was prepared by soaking 2 g of each sample in 100 ml n-hexane for 4 hours twice. The filtrates were discarded and the residue was extracted in 50 ml diethyl ether, filtered into a separating funnel and about 50 ml of the 10% NaOH solution was added. The mixture was shaken very well to separate the aqueous layer from the organic layer, 25 ml distilled water was added; the total aqueous layer was acidified to pH 4.0 by adding 10% HCl solution and 50 ml dichloromethane (DCM). The organic layer was collected, dried and then weighed (Boham & Kocipai, 1994).

Determination of alkaloids

5 g of each sample was weighed into a 250 ml beaker; 200 ml of 20% acetic acid in ethanol was added and allowed to stand for 4 hours. This was then filtered and the extract was concentrated using a water bath to evaporate about a quarter of the original volume. Concentrated ammonia solution was added drop-wise to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, and weighed (Harborne, 1973).

Determination of phytates
4.0g of each sample was soaked in 100ml of 2% HCl for 5hrs and then filtered. 25ml of the filtrate was taken into a conical flask and 5ml of 0.3% ammonium thiocyanate solution (NH_4SCN) was added as an indicator; 53.5ml of distilled water was added to reach pH of 3.5. The mixture was titrated with a ferric chloride solution ($FeCl_3$) until a brownish yellow colour persisted for 5minutes. The result was multiplied by a factor 0.195 and 3.55 to convert to phytate mg/100g (Reddy *et al,* 1982).

Determination of oxalates
75ml of 3.0M H_2SO_4 was added to 1g of ground sample and stirred intermittently with a magnetic stirrer for about one hour and filtered using Whatman No. 42 filter paper. A 25ml sample of the filtrates (extract) was collected and titrated hot (80-90^oC) against 0.05M $KMnO_4$ solution to the point when a faint pink colour appeared that was persistent for at least 30seconds (Jrand & Underwood, 1986; Kayode *et al,* 2011).

$$\text{Oxalate content (mg/100g)} = \frac{T \times [Vme]\,[DF] \times 2.4 \times 10^2}{ME \times Mf}$$

where: T = titre value of $KMnO_4$.
Vme = Volume-mass equivalent (i.e 1ml of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid).
DF = Dilution factor, VT/A
VT = Total volume of filtrate (75ml)
A = Aliquot used (25ml)
ME = molar equivalent of $KMnO_4$
Mf = Weight of sample use**.**

Cyanide content determination
4g of each sample was soaked in a mixture containing 40ml of distilled water and 2ml of orthophosphoric acid. It was then mixed, stoppered and left overnight at room temperature to set free all the bound hydrocyanic acid. The resulting mixture was distilled and about 5ml of distillate was collected into a receiving flask containing 0.1g of NaOH pellets in 40ml of distilled water. The distillate was then transferred into a 50ml volumetric flask and made up to mark with distilled water. 20ml of this was placed in a conical flask and 1.0ml of 5% potassium iodide solution was added and the solution titrated against 0.01M silver nitrate solution. A blank was also titrated until the end point indicates a faint but permanent turbidity (AOAC, 1990).

Results and Discussion
Phytochemical screening of Raphia hookeri and Raphia farinifera

Table 1 The Phytochemical Screening of the shell, pulp and seed of *Raphia hookeri* using methanol, ethylacetate, n-hexane and aqueous extracts.

	Aqueous			**Methanol**			**Ethylacetate**			**n-hexane**		
Phytochemicals	Shell	Pulp	Seed	Shell	Pulp	Seed	Shell	Pulp	Seed	Shell	Pulp	Seed
Saponin	++	+++	+	+	+	+	-	-	-	-	+	-
Terpenoid	-	-	-	++	+++	++	++	+++	++	++	+++	+
Steroids	-	-	-	-	-	-	++	-	++	-	-	-
Cardiac Glycosides	-	-	-	++	+	+	+++	++	+	++	+	+
Flavonoids	++	-	-	-	-	-	+	-	-	+	-	-
Alkaloid	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	++	-	-	++	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	++	-	-	+++	-	++	+++	-	-	+++	++

Key: +++ (abundant), ++ (moderate), + (trace), - (absent)

Tables 1 and 2 present the results of phytochemical screening of aqueous, methanol, ethylacetate and n-hexane extracts of shell, pulp and the seed of *Raphia hookeri* and *Raphia farinifera* fruits. The results show that the aqueous and methanolic extracts are high in saponins, while cardiac glycosides are present in the methanolic, ethyl acetate and hexane extracts of all parts of the plants. Alkaloids and anthocyanins were not detected in any of the extracts while flavonoids were detected only in the shells, tannins were found only in the pulp and steroids were found only in the ethyl acetate extract of *R. hookeri* and hexane extract of *R. farinifera* only.

Table 2 The Phytochemical screening of shell, pulp and seed of *Raphia farinifera* using aqueous, methanol, ethylacetate and n-hexane extracts.

	Aqueous			**Methanol**			**ethylacetate**			**n-hexane**		
Phytochemicals	**Shell**	**Pulp**	**Seed**	**Shell**	**Pulp**	**Seed**	**Shell**	**Pulp**	**Seed**	**Shell**	**Pulp**	**Seed**
Saponin	++	+++	++	+	+	+	-	+	-	-	+	-
Terpenoids	-	-	-	++	+++	++	+	+++	+	++	+++	+
Steroids	-	-	-	-	-	-	-	-	-	+	-	+
Cardiac glycosides	+	_	-	++	+	++	+++	+	+	++	+	+
Flavonoid	+	-	-	-	-	-	++	-	-	+	-	-
Alkaloid	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	++	-	-	++	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	+++	-	-	+++	-	++	+++	-	-	+++	++

Key: +++ (abundant), ++ (moderate), + (trace), - (absent)

The presence of bioactive agents in the fruits will make these plants to have high medicinal values such as anti inflammatory, antioxidant, antimicrobial, antiviral activities.

Quantitative phytochemical composition

Table 3 presents the phytochemical content of *Raphia hookeri* and *Raphia farinifera* fruits.

Table 3 Phytochemical composition (mg/100g) of *Raphia hookeri* and *Raphia farinifera* fruits.

	Raphia hookeri				***Raphia farinifera***			
	***Total phenol**	***Saponins**	***Tannins**	***Flavonoids**	***Total phenol**	***Saponins**	***Tannins**	***Flavonoids**
Shell	3.91± 0.76[a]	0.87± 0.17[a]	ND	1.28 ± 0.07	11.41± 0.89[a]	1.61± 0.27[a]	ND	2.53± 0.10
Pulp	11.51± 0.73[b]	2.25± 0.11[b]	0.60± 0.05	ND	4.52 ± 0.42[b]	5.32± 0.44[b]	1.00± 0.33	ND
Seed	3.59± 0.16[a]	0.79± 0.11[a]	ND	ND	10.44± 1.25[a]	1.25± 0.14[a]	ND	ND

*=results are on wet weight basis.

a,b,..Values are means ± standard deviations of triplicate determinations. Values in the same column sharing different letters are significantly different ($p < 0.05$ level)

Total phenol

The *Raphia hookeri* shell, pulp and seed contained 3.91±0.76, 11.51± 0.73and 3.59±0.016 mg/100 g total phenol respectively. This implies that the pulp of the fruit has the highest total phenol content compared with the shell and seed; while for *Raphia farinifera* the total phenolic content was 11.41±0.89, 4.52±0.42 and 10.44±1.25mg/100g respectively, showing that the pulp has the least total phenol content. Phenols are known to protect plants from oxidative damage and perform the same functions for humans (Okwu, 2005). The outstanding feature of phenols is their ability to block specific enzymes that cause inflammations. They also modify the prostaglandin pathways, thereby protecting platelets from clumping (Okwu & Omodamiro, 2005). Phenolic compounds can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood

vessels (angiogenesis) that is necessary for tumour growth. They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential (Wahle *et al*, 2010).

Saponins

The saponin content was highest in the pulp of both *Raphia hookeri* and *Raphia farinifera*, while the least amount was found in the seeds of both species. Generally, *Raphia farinifera* had higher saponin content than *Raphia hookeri*. Saponins have some general characteristics which include the formation of foams in aqueous solution and hemolytic activities [33]. Saponins have natural tendency to ward off microbes which make them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasions (Okwu, 2004, 2005; Okwu & Omodamiro, 2005; Okwu & Emenike, 2006). These compounds appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells particularly lung and blood cancers (Stray, 1998). They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospect for saponins is how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells, as is the mode of some cancer fighting drugs. Cancer cells have more cholesterol-type compounds on their membranes than normal cells; saponins therefore bind cholesterol and thus interfere with cell growth and division (Okwu, 2005; Okwu & Emenike, 2006).

Tannins: the results from the analysis revealed that only the pulp of *Raphia hookeri* and *Raphia farinifera* fruits contain 0.60±0.05 and 1.00±0.33mg/100g of tannins, respectively. Tannin was not detected in the shell and the seed of the two fruits species. Tannins are plant polyphenolic compounds that are contained in large quantities in food and beverages (tea, red wine, nuts, etc.) consumed by humans daily. It has been shown that various tannins exert broad cancer chemo protective activity in a number of animal models (Nepka *et al*, 1999). Tannins have astringent properties; hasten the healing of wounds, parasitic skin disease and inflamed mucous membranes (Sofowora, 1993; Okwu, 2004; Hutchinson *et al*, 1993).

Flavonoids

The flavonoids contents of *Raphia hookeri* and *Raphia farinifera* shell are 1.28±0.07 and 2.53±0.10 respectively. Flavonoids, however, were not detected in the pulp and seed of the two species. The amounts of flavonoids present in the shell of *Raphia farinifera* are higher than that of *Raphia hookeri*. The biological functions of flavonoids include protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor (Okwu, 2004).

Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis. Flavonoids in the intestine lower the risk of heart diseases (Okwu, 2004).

Conclusion

This study has shown that *Raphia hookeri* and *Raphia farinifera* fruits contain bioactive phytochemicals, thus giving credence to the medicinal use of the fruits. It will be necessary however, to determine the nutritional contents of the fruits as well as the effect of processing on both the nutritional and phytochemical contents.

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