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Phytochemical characterization and antioxidant potential of *Raphia hookeri* mesocarp from Mebole (Gabon)

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Abstract
This study presents the results of antioxidant potential and phytochemical analysis of mesocarp fruits of *Raphia hookeri*. Phytochemical characterization was performed on extracts obtained by polarity gradient with the following solvents: ethyl acetate, acetone, and methanol. The evaluation of the antioxidant potential was performed with the aqueous extract. The results obtained indicate the abundance of alkaloids, polyphenols, triterpenes, and reducing compounds in each extract. However, coumarins, anthracene compounds, flavanones, and flavanols were absent in each extract. Leucoanthocyanins are present in the ethyl acetate extract and the methanolic extract. Flavones are present in the acetone extract and the methanolic extract. Free anthracenes, saponins, catechin tannins, and catechins were present in the methanolic extract. Gallic tannins were present in the acetone extract. The antioxidant activity results were very low: IC_{50} = 521.43 ± 1.02 µg/mL; AAI = 0.1 ± 0.0001.

Keywords: *Raphia hookeri*, Mesocarp, Antioxidant activity index, phytochemical composition, Inhibition concentration (IC_{50})

Introduction
The Raphia palm belongs to the *Palmaceae* family [1]. It grows naturally in swampy, wooded areas and along the coastline [2]. Many studies have associated fruit and vegetable consumption with a minor incidence of cardiovascular disease, cancer, and other diseases [3]. In contrast, the chemical composition of fruits differs from one fruit to another, and it is important to favor fruits containing more bioactive compounds [3]. The investigations on Raphia palms have shown that the mesocarp of the ripe Raphia fruit produces edible oil [4]. Similarly, it has been reported that the cooked mesocarp is edible and that it has medicinal properties [4]. However, there exists no information about the therapeutic potential of the *Raphia hookeri* mesocarp from Gabon, particularly on the antioxidant activity. The interest of this work is to evaluate the phytochemical composition of the mesocarp of *Raphia hookeri* fruit and then to assess the antioxidant activity of the aqueous extract of mesocarp.

Materials and methods
Plant material
Raphia hookeri's fruits were amassed in the Mebole Village (Gabon). The fruits were transported to the laboratory in bags and then the mesocarps were removed and dried in the oven at 90 °C for 24 h. The dried mesocarps were powdered using the mechanical grinder. The powder obtained was kept in the refrigerator at 4 °C. The National Herbarium of Gabon Pharmacopoeia Institute of Traditional Medicine (Iphametra) of Libreville allowed identifying the plant.

Preparation of extract
Extraction was achieved using the protocol described by Koffi N'guessan with few modifications [5]. 150mL of cyclohexane was summed to 30g of powder. The mix is agitated for 24h to room air. After separation through Whatman No. 1 filter paper, the residue is dried in the oven at 80 °C aimed at 24h and the filtrate was concentrated in rotavapor to give oil with 43% yield. To the dried residue achieved, is added 150mL of ethyl acetate, and the mix is stirred to room air aimed at 24h. After filtration through Whatman No. 1 filter paper, the residue was again made dry on an oven at 80 °C for 24 h and the filtrate (Ethyl acetate extract) was kept at 4 °C until analysis. To the new residue, was added 150 mL of acetone and the mixture was stirred at room temperature for 24 h.

After filtration through Whatman No. 1 filter paper, the residue is made dry on the oven at 80 °C for 24h and the filtrate (Acetonic extract) is kept at 4 °C until analysis. In the finish, the residual marcs were added to methanol. The mixture was agitated for 24h to room air and filtrated through Whatman No. 1 filter paper allowed to the Methanolic extract. To 30 g of dried powder of mesocarp was added 150mL of distilled water. The mix is heated in reflux for 1h. After cooling to room air and filtration through Whatman No. 1 filter paper, the aqueous extract [6] is lyophilized and stowed in the refrigerator at 4 °C until the evaluation of the antioxidant activity.

Phytochemical analyses

Standard procedures with small modifications are used for the identification of phytochemicals. The extract is tested for the presence of flavonoids, tannins, polyphenols, reducing compounds, alkaloids, saponins, coumarins, sterols, and triterpenes.

- **Polyphenols**

2mL of filtrate was mixed with 1mL of Folin-ciocalteu reagent and 1mL of sodium bicarbonate (Na_2CO_3). Polyphenols are identified by the dark green staining [7].

- **Tannins**

3 mL of extract solution with 1 mL of 10% lead acetate is introduced into a test tube. The appearance of a white precipitate indicates a positive test for tannin identification. For differentiation of tannins, 2 mL of extract solution with 2 mL of 1% copper sulfate is added to a test tube. Two drops of ammonia are added to the test tube. The appearance of a blue precipitate indicates a positive test for the identification of gall tannins. On the other hand, a green precipitate indicates a positive test for the identification of catechin tannins [5].

- **Alkaloids**

2 mL of extract solution with a few drops of Dragendorff's reagent are introduced into a test tube. The appearance of an orange-red precipitate reveals a positive test for the identification of alkaloids [8].

- **Flavonoids**

5 mL of extract solution with 5 mL of hydrochloric ethanol (95° ethanol, distilled water, and hydrochloric acid R in equal volumes of 5 mL) are introduced into a test tube. A few magnesium chips with 1 ml of isoamyl alcohol are added to the test tube. The test is positive when one of the following colors is seen on the isoamyl alcohol supernatant layer:

- A pink-orange color (flavones).
- A pink-purple color (flavanones).
- A red color (flavonols and flavanonols).

Without magnesium chips in the cyanidin reaction and by placing in a water bath for 10 minutes, the test is positive for leuco-anthocyanins by the appearance of the cherry-red color and positive for catechols for a brown-red color [10].

- **Triterpenoids**

2 mL of extract solution with a few drops of concentrated sulfuric acid are introduced into a test tube. The appearance of a purple coloration indicates a positive test for the identification of triterpenes [10].

- **Coumarins**

2mL of extract solution with 3mL of 10% sodium hydroxide solution. The appearance of yellow color after handshaking of the mixture indicates a positive test for coumarin identification [10].

- **Saponins**

2 mL of extract solution with 5 mL of distilled water are introduced into a test tube. The resulting mixture is shaken vigorously by hand (lengthwise) for 15 seconds. The observation of a persistent foam for 20 min or a froth indicates a positive test for the identification of saponins in the tested extract [11].

- **Reducing compounds**

2 mL of extract solution with 1mL of Fehling's liquor is introduced into a test tube. The mixture is left in a boiling water bath for 15 minutes. A brick-red precipitate reveals a positive test for the identification of reducing compounds [5].

- **Anthracenes**

1 mL of extract solution with 1 mL of dilute ammonium hydroxide (NH_4OH) solution is added to a test tube. A red color after handshaking of the mixture indicates a positive test for free anthraquinones [10].

5 mL of extract solution with 5 mL of petroleum ether are introduced into a separatory funnel. The mixture is stirred, then the organic phase is withdrawn and introduced into a test tube. 1 mL of diluted ammonium hydroxide solution (NH_4OH) is added to the test tube. Then the mixture in the test tube is shaken by hand and at the appearance of the intense red color reveals a positive test for the identification of combined anthraquinones [10].

Evaluation of the antioxidant activity

The Antioxidant Activity Index (AAI) is determined by the method described by Scherer and Godoy [12-13] with small modifications. This method is based on the DPPH (2, 2-diphenyl-2- picrylhydrazyl) radical test. A standard solution of DPPH of concentration 100 µg/mL is prepared in ethanol as well as different concentrations of lyophilized extracts ranging from 0.25 to 100 µg/mL in ethanol. 1 mL of DPPH standard solution is mixed with 1 mL of each extract solution. The absorbance is measured at 517 nm using a Genesys 10 UV-Visible spectrophotometer against a control (1 mL DPPH with 1 mL ethanol) after 15 minutes of incubation at room temperature and in the dark. Ascorbic acid (vitamin C) is used as a reference. The measurements are performed in triplicate. The antiradical activity is calculated using the following equation

$$RSA = \frac{\text{Abs(control) - Abs(sample)}}{\text{Abs(control)}} * 100$$

RSA is the percentage of free radical scavenging activity, Abs (control) the absorbance of DPPH radical + ethanol, and Abs (sample) the absorbance of DPPH radical + sample extract or standard. The antioxidant activity is expressed as IC_{50} which is the measure of concentration in µg/mL of extract that inhibits 50 % of DPPH radicals. The antioxidant activity index was evaluated by using the following formula:

$$AAI = \frac{[\text{DPPH}(\mu g/mL)]}{IC_{50}\ (\mu g/mL)}$$

[DPPH (µg/mL)] is the final concentration of DPPH

Results and Discussions

The results of the phytochemical composition of the crude extracts of the mesocarps of *Raphia hookeri* fruits are recorded in Table 1.

Table 1: Results of phytochemical screening

Phytochemicals		Ethyl acetate extract	Acetonic extract	Methanolic extract
Polyphenols		+	++	++
Tannins	Gallic	+	++	-
	Catechin	+	-	++
Alkaloids		+	++	++
Flavonoids	Flavones	-	+	++
	Flavanones	-	-	-
	Flavonols	-	-	-
	Leucoanthocyans	+	++	-
	Catechols	-	-	++
Saponins		-	-	+
Triterpenes		+	++	++
Reducing Compounds		++	+	++
Coumarins		-	-	-
Anthracenes	Combinations	-	-	-
	Free	-	-	+

(++) abundant; (+) present; (-) absent

These results reveal the presence of polyphenols, alkaloids, triterpenes, and Reducing Compounds in all extracts. However, coumarins, combinations of anthracenes, flavanones, and flavanols were absent in all extracts. Leucoanthocyans are present in the ethyl acetate extract and the acetonic extract. However, flavones are present in the acetonic extract and the methanolic extract. Free anthracenes, saponins, and catechols were present in the methanolic extract. However, the gallic tannins were present in the acetonic extract and in the ethyl acetate extract. Or the catechin tannins were present in the methanolic extract and in the ethyl acetate extract. Contrary to the results achieved in Nigeria by Oluwaniyi *et al*. (2014) [4], alkaloids, anthocyanins, and flavonoids are absent in methanolic extract and ethyl acetate extract. However, coumarins are present in these extracts. This difference could be explained by environmental, climatic, and seasonal factors [5]. The presence of saponins, free anthracenes, and catechol in methanolic extract shows that these compounds are soluble only in this solvent. Likewise, gallic tannins are present in the ethyl acetate extract and in the acetonic extract. Indeed, the nature of the solvent influences the extraction of organic compounds [13]. Nevertheless, the compounds identified in only one extract are of low quantity or the compounds identified in two extracts are in moderate quantity in the mesocarps of *Raphia hookeri* fruits. The absence of leucoanthocyans in methanolic extract shows that the quantity of leucoanthocyans is moderate in the mesocarps of *Raphia hookeri* fruits. Because all leucoanthocyans were extracted by ethyl acetate and acetone. The absence of flavones in ethyl acetate extract suggests that flavones are not soluble in ethyl acetate solvent and moderate amounts in the mesocarps of *Raphia hookeri* fruits. The abundance of secondary metabolites like reducing compounds, polyphenols, alkaloids, and triterpenes can give many biological properties to the mesocarps of *Raphia hookeri* fruits [15]. Indeed, Polyphenols are antioxidants, anti-inflammatories, and anti-hypertensives; Alkaloids have analgesic, antidiabetic, anticancer, anti-microbial, anti-tumor, and anti-parasitic effects; tannins are antibacterial, antioxidants, anti-inflammatories, anti-diabetics; Triterpenes can fight inflammation. Finally, leucoanthocyanes have a diuretic (antihypertensive) action. The presence of these phytochemicals in the mesocarps of *Raphia hookeri* fruits extracts could prevent risks against pathologies such as cardiovascular diseases, hypertension, diabetes, and cancer…

The results of the assessment of the antioxidant activity by the anti-free radical activity index (AAI) method are consigned in table 2.

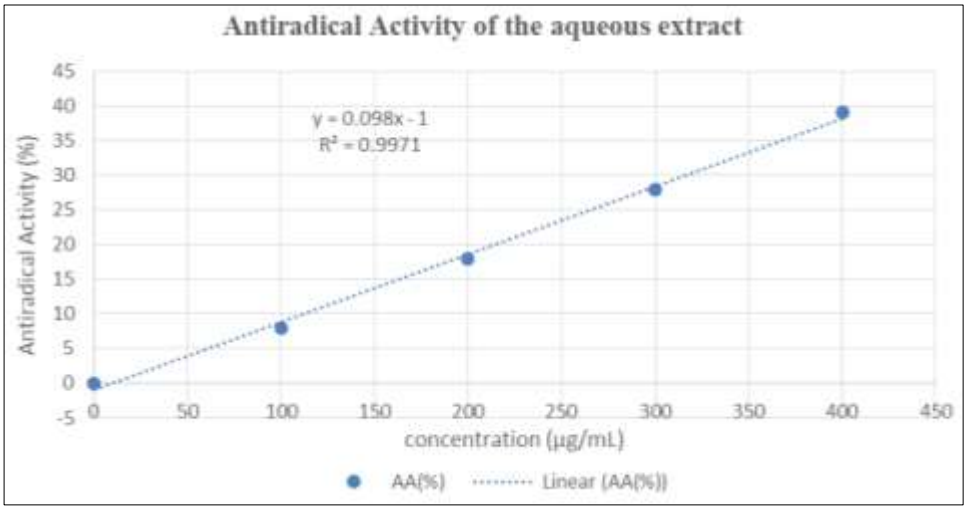

Fig 1: Antiradical activity curve of the aqueous extract of mesocarps of *Raphia hookeri* fruits

Table 2: Results of the evaluation of antioxidant activity.

	Equation	R^2	IC_{50}	AAI
Plant material	Y= 0.098X-1	0.997	521.43±1.02	0.1 ±0.0001
Ascorbic acid (standard)	Y= 5.120X + 01.46	0.971	4.89 ± 2.8	10.22 ± 0.9

The index of the antiradical activity of the aqueous extract of mesocarps of *Raphia hookeri* fruits was 0.1 ±0.0001. According to this index, the antioxidant activity is considered low if AAI < 0.5, moderate if 0.5 <AAI < 1, high if 1 <AAI < 2 and very high if AAI > 2 [5, 11]. The antioxidant activity of the aqueous extract of mesocarps of *Raphia hookeri* fruits is then low despite the abundance of polyphenols. This result could be explained by the absence in the aqueous extract of some classes of flavonoids and gallic tannins responsible for the high antioxidant activity [6]. In this aqueous extract, the antioxidant activity would probably be due to catechin tannins and free anthracenes [6]. Our results on the antioxidant activity of mesocarp of *Raphia hookeri* fruit are different from those found in Cameroon by Eric Serge Ngangoum *et al*. (2019) [16]. Indeed, their study shows that the mesocarp of *Raphia hookeri* fruit has a strong antioxidant activity (very close to vitamin C). This difference can be explained using alcoholic extracts (methanolic and ethanolic) in the evaluation of their antioxidant activity.

Conclusion

The phytochemical characterization of the extracts of *Raphia hookeri* mesocarp detected polyphenols, alkaloids, tannins (gallic and catechin), reducing compounds, triterpenes, Flavonoids (flavones, leucoanthocyans, and catechol), saponins, and free anthracenes. These compounds are renowned for their many biological properties. However, coumarins, combination anthracenes, flavanones, and flavanols are absent. Despite the low antioxidant activity of the aqueous extract attributed to the absence of many groups of flavonoids and gallic tannins, the use of mesocarp of *Raphia hookeri* fruit in the diet or herbal medicine could protect populations against many diseases.

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