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## Phytochemical composition, total polyphenols and total flavonoids content of the aqueous extract of *Raphia hookeri* mesocarp from Mebole village (Gabon)

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### Abstract

The *Raphia hookeri* is a palm tree that grows naturally in marshy and wooded areas and thus the origin extends from Central Africa to West Africa. Cooked Mesocarp of the *Raphia hookeri* is exploiting in the diet of Gabonese populations. The present study aims to determine the phytochemical composition, quantify the total polyphenols and total flavonoids content of the aqueous extract made by decoction of the dried pulp powder. A phytochemical screening achieved on this aqueous extract simply revealed the presence of polyphenols, catechic tannins, leucoanthocyanins, saponins, triterpenes, reducing compounds, coumarins, free anthracenes, and alkaloids. An assay of total polyphenols and total flavonoids was too achieved and the results indicated that mesocarps of fruits of *Raphia hookeri* are rich in total polyphenols ( $631.70 \pm 4.38$  mg GEA/ 100g) and total flavonoids ( $92.07 \pm 2, 72$  mg EQ/ 100g).

**Keywords:** *Raphia hookeri*, aqueous extract, phytochemistry, polyphenols, flavonoids

### Introduction

Raphia is a plant belonging to the *Palmaceae* family <sup>[1]</sup> that grows in swampy and semi-swampy environments of the equatorial rain forest or derived savannas <sup>[2]</sup>. All parts of the *Raphia hookeri* palm tree have an economic value <sup>[3]</sup>. Indeed, the cooked mesocarp of the ripe Raphia fruit is edible and produces vegetable oil. The pulp of the fruit can be fermented into an alcoholic beverage <sup>[4]</sup> in the same way that the palm tree can be bled for sap, which is fermented into palm wine <sup>[5]</sup>. The leaves of the Raphia palm tree are used as a building material (roofing and ceiling) and as a source of fiber used in the manufacture of bags, wallets, shoes, cases, furniture, decorations, and several works of art <sup>[3]</sup>. To contribute to the valorization of local edible products, the objective of this study is to analyze the aqueous extract of the dried powder of the mesocarp of the ripe Raphia fruit. Phytochemical screening of the extract was realized to determinate the mains secondary metabolites. This analysis was accompanied by quantification of total polyphenols and total flavonoids.

### Materials and Methods

#### Plant material

*Raphia hookeri* fruits were collected from Mebolo village in the province of woleu ntem, Gabon. The fruits were transferred to the laboratory in different polyethylene bags. after separating the three parts of the fruit (i.e shell, pulp, and the seed), The mesocarps were then dried at the oven at 90 °C for 24 h and pulverized to powder using the mechanical grinder. The powder achieved was stored in the refrigerator at 4 °C. The plant was identified at the National Herbarium of Gabon Pharmacopoeia Institute of Traditional Medicine (Iphametra) of Libreville.

#### Preparation of extract

Extraction was carried out by decoction using 30 g of the mesocarp powder in 150 mL of distilled water. The mixture was boiled under reflux for 1 h. After cooling at room temperature, the mixture was strained through Whatman No. 1 filter paper. The filtrate achieved was stored at 4 °C until analyses.

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### Phytochemical analyses

Standard procedures with small modifications were used in the qualitative determination of the phytochemicals. The extract was tested for the presence of polyphenols, flavonoids, tannins, alkaloids, saponins, coumarins, reducing compounds, sterols, and triterpenes.

#### • Polyphenols

To 2 mL of filtrate was added 1 mL Folin-ciocalteu reagent and 1mL sodium bicarbonate ( $\text{Na}_2\text{CO}_3$ ). Dark- green coloration indicated the presence of polyphenols [6].

#### • Tannins

To 3 mL of filtrate was added 1 mL of 10% lead acetate solution. White precipitate indicated the presence of tannins. For differentiation of tannins, 2 mL of filtrate, 2 mL of 1% copper sulfate solution and 2 drops of ammonia were mixed. Blue precipitate indicated the presence of gallic tannins and green precipitate catechic tannins [7].

#### • Alkaloids

To 2 mL of filtrate were additional a few drops of Dragendorff reagent. A reddish-orange precipitate or coloration indicated the presence of alkaloids [8].

#### • Flavonoids

To 4 mL of filtrate was added 1 mL of 1% ammonia solution. Yellow coloration indicated the presence of flavonoids [7]. Combination of 5 mL of filtrate with 5 mL of hydrochloric ethanolic solution added to 1 mL of isoamylic alcohol and some magnesium chips was prepared. Development of orange pink coloration indicated the presence of flavones. Flavanones were detected by purplish pink coloration. Red coloration revealed the presence of flavonols [9]. The cyanidine reaction without magnesium and after heating for 10 minutes in the water bath showed a cherry-red color indicating the presence of leucoanthocyan [8].

#### • Sterols and triterpenoids

To 2 mL of filtrate were additional a few drops of concentrated sulfuric acid. We observed appearance of a purple coloration in the presence of triterpenes and green coloration for sterols [10].

#### • Coumarins

To 2 mL of filtrate was added 3 mL of 10% sodium hydroxide solution. After shaking the mixture, the appearance of a yellow color indicated the presence of coumarins [10].

#### • Saponins

The mixture of 2 mL of filtrate and 3 mL of distilled water was shaken vigorously for 15 seconds. The observation of persistent foam for 20 minutes indicated the presence of saponins in the extract [11].

#### • Reducing compounds

The mixture of 2 mL of filtrate and 1 mL of Fehling's liqueur

was heated in a water bath for 15 minutes. The appearance of a brick-red precipitate indicated the presence of reducing compounds [7].

### Determination of total polyphenols and total flavonoids

#### • Total polyphenols

The assessment of total polyphenols was achieved by the method of Folin-Ciocalteu [12] on lyophilized extract. 1mL of Folin-Ciocalteu reagent (0.2 N diluted in MeOH) was mixed with 200  $\mu\text{L}$  of extract dissolved in distilled water (1 mg/mL). After 5 min incubation in dark at room temperature, 800  $\mu\text{L}$  of 20% sodium bicarbonate solution (w/v) was added. Sample was incubated at room temperature for 1 h. Absorbances were determined at 765 nm using a GENESYS 10 UV spectrophotometer. A reagent blank extract with MeOH was prepared. All tests were achieved in triplicate. The total polyphenol content was assessed using a reference curve performed with gallic acid (0-150 mg/L). The results were expressed in mg of Gallic Acid Equivalent (GAE) per 100 g dry extract according to the following formula:

$$C = (Cl \times D \times 10/M) \times 100$$

C: Concentration of the sample in  $\mu\text{g}$  GAE/100 mg dry extract.

Cl: sample concentration in  $\mu\text{g}$  GAE/mL.

D: dilution factor

M: sample mass (mg).

#### • Total Flavonoids

Total flavonoids were determined according to the method described by Arvouet-Grant [12] with small modifications. 1 mL of 2%  $\text{AlCl}_3$  in methanol was mixed with 1 mL of lyophilized extract diluted in methanol (1 mg/mL) for 10 min. The absorbances were determined at 415 nm using a GENESYS 10 UV spectrophotometer. A reagent blank with 2 mL of methanol was prepared. Quercetin was used as a standard for establish the standard range (0-50 mg/L). The results are expressed as mg Quercetin Equivalent (QE) per 100 g dry extract and calculated according to the following formula:

$$C = (Cl \times D \times 10/M) \times 100$$

C: Concentration of the sample in  $\mu\text{g}$  QE/100 mg dry extract.

Cl: sample concentration in  $\mu\text{g}$  QE/mL.

D: dilution factor.

M: sample mass (mg).

### Results and Discussions

The phytochemical analysis of the aqueous extract of the mesocarps of *Raphia hookeri* was carried out to identify the major organic chemical compounds occurring in the extract. The results in table 1 reveal the present of polyphenols, catechic tannins, leucoanthocyan, saponins, triterpenes, reducing compounds, coumarins, free anthracenes, and alkaloids. However, we note the absence of gallic tannins, flavones, flavanones, flavonols, catechols, sterols, and combination anthracenes.

**Table 1:** Results of phytochemical screening of aqueous extracts of *Raphia hookeri*

| Organic Compounds |          | Results |
|-------------------|----------|---------|
| Polyphenols       |          | ++      |
| Tannins           | Gallic   | -       |
|                   | Catechic | ++      |
| Alkaloids         |          | ++      |

|                        |                |    |
|------------------------|----------------|----|
| Flavonoids             | Flavones       | -  |
|                        | Flavanones     | -  |
|                        | Flavonols      | -  |
|                        | Leucoanthocyan | ++ |
|                        | Catechols      | -  |
| Saponins               |                | +  |
| Sterols et triterpenes | Sterols        | -  |
|                        | Triterpenes    | +  |
| Reducing Compounds     |                | +  |
| Anthracenes            | Combinations   | -  |
|                        | Free           | +  |
| Coumarins              |                | ++ |

(++) abundance; (+) presence; (-) absence

These results are different from those obtained by Oluwaniyi *et al.* (2014) [3]. Because in their study, we note the Absence of alkaloids, Terpenoids, and anthocyanin [3]. Indeed, the phytochemical composition of plants depends on many environmental factors [12]. The abundance of polyphenols, alkaloids, leucoanthocyan, coumarins and catechic tannins can give many biological properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antibacterial, antitumor, anti-parasitic, analgesic, antidiarrheal, antiseptic, antihypertensive, etc. [13-18]. Indeed, Polyphenols have known

for their antioxidant activity [13], alkaloids for their analgesic power [13], tannins for their antibacterial effect [19-20], leucoanthocyan for their diuretic (antihypertensive) action [21], and finally coumarins for their antimicrobial activity [22]. The abundance of antioxidant compounds insinuates that the consumption of the pulp of fruits (*Raphia hookeri*) can avoid many diseases like cancer, cardiovascular diseases, and diabetes [7].

The results of total polyphenols and total flavonoids evaluate are shown in Tables 2 & 3.

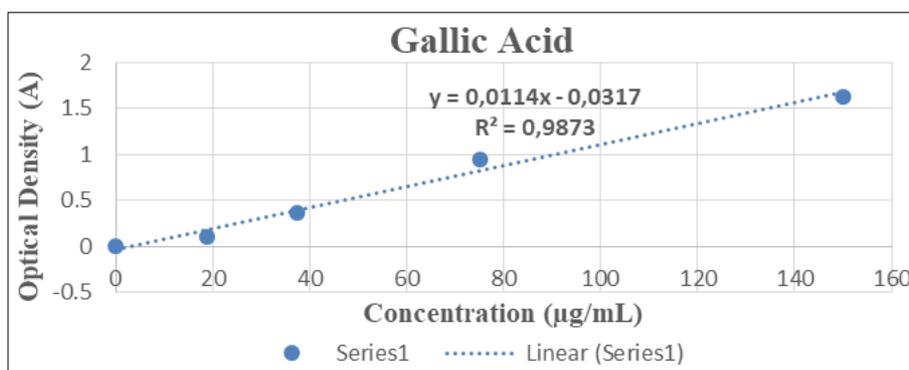


Fig 1: Gallic acid standard curve for total polyphenols content

Table 2: Result of the determination of total polyphenols in the aqueous

| Test                | Total Polyphenols               |
|---------------------|---------------------------------|
| Optical Density (A) | Concentration (mg GAE/ 100g MS) |
| 0.150               | 637.54                          |
| 0.148               | 630.53                          |
| 0.147               | 627.02                          |
| average             | 631.70                          |
| Standard deviation  | 4.38                            |

The total polyphenols content was  $631.70 \pm 4.38$  mg GAE/100g of dry matter and the total flavonoids content was  $92.07 \pm 2$ , 72 mg QE/100g of dry matter. These values are tallest by comparison with data issued in the literature [3] and validate the results of the phytochemical analyses. In comparison to total polyphenols content of fruits and vegetables such as strawberry (263.8 mg GAE/100g), Litchi (222.3 mg GAE/100g), grape (195.5 mg GAE/100g), datte (99.3 mg GAE/100g) or artichoke (heart, 321.3 mg GAE/100g), pulp of the fruits of *Raphia hookeri* is very rich in total polyphenols and total flavonoids [12, 23].

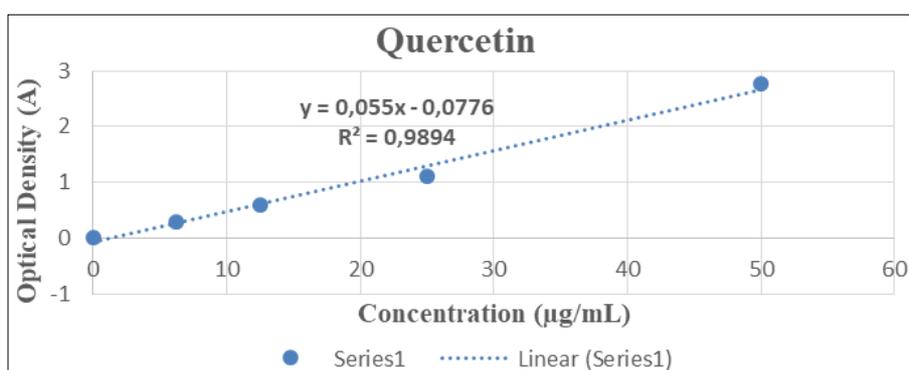


Fig 2: Quercetin standard curve for total flavonoids content

**Table 3:** Result of the determination of total flavonoids in the aqueous extract

| Test                | Total Flavonoids               |
|---------------------|--------------------------------|
| Optical Density (A) | Concentration (mg QE/ 100g MS) |
| 0.045               | 89.16                          |
| 0.048               | 91.35                          |
| 0.054               | 95.71                          |
| average             | 92.07                          |
| Standard deviation  | 2,72                           |

### Conclusion

Phytochemical studies revealed that the aqueous extract of pulp of the fruits of *Raphia hookeri* contained polyphenols, catechic tannins, leucoanthocyanins, saponins, triterpenes, reducing compounds, coumarins, free anthracenes, and alkaloids. In contrast, gallic tannins, flavones, flavanones, flavonols, catechols, sterols, and combination anthracenes are absent. The abundance of polyphenols noted in phytochemistry screening has confirmed by the results of the quantitative analysis of total polyphenols ( $631.70 \pm 4.38$  mg GAE / 100g of dry matter) and total flavonoids ( $92.07 \pm 2, 72$  mg QE/ 100g of dry matter). These results show that the mesocarps of the studied plant are rich in organic compounds that can prevent human beings from many diseases such as diarrhea, cancer, hypertension, diabetes, etc.

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