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Phytochemical characterization and of total polyphenols and flavonoids content of the aqueous extract of the seeds of *Cucumeropsis edulis* (Cucurbitaceae) from Gabon.

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Abstract
Cucumeropsis edulis (Cucurbitaceae) is a plant cultivated in many countries of Central Africa. Its seeds are using in the diet of Gabonese population. The present study consists to analyse the phytochemical composition, quantify polyphenols and flavonoids content of the aqueous extract prepared by decoction of the powder of the dried seeds. A phytochemical screening performed on this aqueous extract showed only the presence of flavonoids, polyphenols, alkaloids and gallic tannins. An assay of total polyphenols and total flavonoids was also carried out. The results showed that the seeds are rich in polyphenols (257 ± 0,004 mg GEA/ 100g) and flavonoids (215 ± 0,02 mg QE/ 100g).

Keywords: *Cucumeropsis edulis*, phytochemistry, polyphenols, flavonoids

Introduction
In Gabon, human beings are used plants for food, medicin, and other purposes. The seeds of *Cucumeropsis edulis* (Cucurbitaceae) commonly known as cucumber are used in the diet of many people [1, 2]. Its fruits with a cylindrical shape contain many small flat and oval seeds [1,3]. These seeds, when roasted and crushed, can be used to thicken sauces, to make a paste and to extract vegetable oil [3, 4]. Despite many use, phytochemical constituents linked to the biological properties of these seeds are not known in Gabon. To contribute to the valorization of local edible products, the objectif of this study consists to analyse the aqueous extract of the dried powder of the seeds of *Cucumeropsis edulis*. Phytochemical screening of the extract was performed to determinate the mains secondary metabolites. This analysis was followed by quantification of total polyphenols and total flavonoids.

Materials and methods
Plant material
The fruits of *Cucumeropsis edulis* were harvested in September 2018 at the village Momo at 50 km of Oyem (Gabon). The seeds with shells were dried at room temperature and under the sunlight. After extraction of shells, the seeds were dried on oven at 90°C for 24 h and pulverized to powder using the mechanical grinder. The powder obtained was stored in the refrigerator at 4°C. The plant was authenticated at the National Herbarium of Gabon Pharmacopoeia Institute of Traditional Medicine (IPHAMETRA) of Libreville.

Preparation of extract
150 mL of distilled water was added to 30 g of the seeds powder. The mixture was heated under reflux for 1 h. After cooling at room temperature, the mixture was filtered through Whatmann No. 1 filter paper. The filtrate obtained was stored at 4°C until analyses.

Phytochemical analyses
Phytochemical screening is a qualitative analysis, based on staining or precipitation reactions, to identify organic compounds called secondary metabolites. The analyses were released using standard procedures with small modifications. 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 (Na_2CO_3). Dark- green coloration indicated the presence of polyphenols [5].

- ***Tannins***

To 3 mL of filtrate was added 1 mL of 10% lead acetate solution. White precipitate indicated the presence of tannins [6]. 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 [4].

- ***Alkaloids***

To 2 mL of filtrate were added a few drops of Dragendorff reagent. A reddish-orange precipitate or coloration indicated the presence of alkaloids [7].

- ***Flavonoids***

To 4 mL of filtrate was added 1 mL of 1% ammonia solution. Yellow coloration indicated the presence of flavonoids [8]. The mixture of 5 mL of filtrate, 5 mL of hydrochloric ethanolic solution, 1 mL of isoamylic alcohol and some magnesium chips was prepared. Appearance of orange pink coloration indicated the presence of flavones. Flavanones were detected by purplish pink coloration. Red coloration indicated 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 leucoanthocyans [7].

- ***Sterols and triterpenoids***

To 2 mL of filtrate were added 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 [12].

Determination of total polyphenols and total flavonoids

- ***Total polyphenols***

The determination of total polyphenols was performed 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 µL of extract dissolved in distilled water (1 mg/mL). After 5 min incubation in dark at room temperature, 800 µL of 20% sodium bicarbonate solution (w/v) was added. Sample was incubated at room temperature for 1 h. Absorbances were measured at 765 nm using a GENESYS 10 UV spectrophotometer. A reagent blank extract with MeOH was prepared. All tests were performed in triplicate. The total polyphenol content was measured using a reference curve performed with gallic acid (0-200 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 µg GAE/100 mg dry extract.
Cl: sample concentration in µg GAE/mL.
D: dilution factor.
M: sample mass (mg).

- ***Total Flavonoids***

Total flavonoids were determined according to the method described by Arvouet-Grant [13] with small modifications. 1 mL of 2% $AlCl_3$ in methanol was mixed with 1 mL of lyophilized extract diluted in methanol (1 mg/mL) for 10 min. The absorbances were measured 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 (0-200 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 µg QE/100 mg dry extract.
Cl: sample concentration in µg QE/mL.
D: dilution factor.
M: sample mass (mg).

Results and Discussions

The phytochemical screening of the aqueous extract of the seeds of *Cucumeropsis edulis* was released to detect the major organic chemical compounds occurring in the extract. The results show the present of polyphenols, gallic tannins, leucoanthocyans and alkaloids (Table 1). However, we note the absence of catechic tannins, flavones, flavanones, flavonols, reducing compounds, saponins, coumarins, sterols and triterpenes. These results are different from those found by Yété pélagie *et al*. in 2015 [14] because in their study, we note the presence of coumarins, saponins, reducing compounds, and catechic tannins [14, 15]. Indeed, the phytochemical composition of plants depends on many environmental factors.

The abundance of polyphenols, alkaloids and gallic tannins can give many biological properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antibacterial, anti-tumor, anti-parasitic, analgesic, antidiarrheal, antiseptic, antihypertensive, etc [14, 16-20]. Polyphenols have known for their antioxidant activity [14], alkaloids for their analgesic power [14], tannins for their antibacterial effect [21, 22] and finally leucoanthocyans for their diuretic (antihypertensive) action [23]. The abundance of antioxidant compounds suggests that the consumption of the foods done with these seeds can prevent many diseases like cancer, cardiovascular diseases, and diabetes.

Table 1: Results of phytochemical screening of aqueous extracts of cucumeropsis edulis

Organic Compounds		Results
Polyphenols		++
Tannins	Gallic	++
	Catechic	-
Alkaloids		++
Flavonoids	Flavones	-
	Flavanones	-
	Flavonols	-
	Leucoanthocyans	+
Reducing Compounds		-
Saponosides		-
Sterols et Triterpenes	Sterols	-
	Triterpenes	-
Coumarines		-

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

The results of total polyphenols and total flavonoids assay are shown in Table 2. The total polyphenol content is 293 mg GAE/100g of dry matter and the total flavonoid content is 219 mg EQ/100g of dry matter. These values are highest by comparison with data published in the literature [14] and confirm 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), seeds of *Cucumeropsis edulis* are very rich in polyphenols and flavonoids [14, 24].

Table 2: Total Polyphenols and Total Flavonoids Assay Results

	Total polyphenols (mg GAE/ 100g)	**Total flavonoids (mg QE/ 100g)**
Results	293 ±0,02	219±0,03
Equation	Y= 0.0114x - 0.0317	Y= 0.055x - 0.0776
Correlation coefficient R^2	0.9873	0.9894

Conclusion

Phytochemical studies showed that the aqueous extract of seeds of *Cucumeropsis edulis* contained leucoanthocyans, polyphenols, alkaloids and gallic tannins. Catechic tannins, flavones, flavanones, flavonols, reducing compounds, saponins, coumarins, sterols and triterpenes are absent. The abundance of polyphenols observed in phytochemistry screening has confirmed by the results of the quantitative analysis of total polyphenols (293 ± 0.02 mg GAE / 100g of dry matter) and flavonoids (219 ± 0.03 mg QE/ 100g of dry matter). These results show that seeds of the plant studied in rich in organic compounds which can prevent human being to many diseases like diarrhea, cancer, hypertension, diabetes, etc.

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