



RESEARCH ARTICLE

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF THE SEEDS OF *CUCUMEROPSIS EDULIS* (CUCURBITACEAE) FROM MOMO IN GABON

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Abstract

This paper describes results of phytochemical analyses and antioxidant activity of the seeds of *Cucumeropsis edulis*. For phytochemical analyses, extraction was performed with the same powder using different solvents: Acetone the first, ethyl acetate the second and methanol the last. Aqueous extract was used for evaluation of antioxidant activity. The results obtained show the abundance of polyphenols, gallic tannins and alkaloids in all extracts. Reducing compounds are present in ethyl acetate and acetonitrile extracts and leucoanthocyanins are present in methanolic extract only. The results ($IC_{50} = 2249 \pm 87.03 \mu\text{g/mL}$; $AAI = 0.022 \pm 0.001$) of evaluation of antioxidant activity are very low.

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Introduction:-

Cucumeropsis edulis (Cucurbitaceae) commonly called cucumber for many people in Africa (Savadogo et al., 2011; pl@nte use, 2020) is a plant used for food, medicine and other purposes. Its leaves are lobed and convenient to purge infants (Raponda and Sillans, 1995). The fruits are cylindrical and contain many small, flat and oval seeds (Savadogo et al., 2011; Raponda and Sillans, 1995). These seeds, when roasted and crushed, are used to prepare many dishes and to extract vegetable oil (Raponda and Sillans, 1995; Adodo et al., 2015; Ndola, 2015). Consumption of vegetables is recommended for the good health of human beings. Indeed, vegetables contain essential elements needed by organism such as secondary metabolites which are many biological properties: anticancer, antidiabetic, antihypertensive, anti-inflammatory, analgesic, antioxidant, etc. (Yété et al., 2015; Mangambu et al., 2014; Adaramoye et al., 2005). Studies into free radicals have shown that foods rich in antioxidants can prevent cardiovascular diseases, cancers and neurodegenerative diseases (Narenda et al., 2010). Despite the consumption of food prepared by the seeds of *Cucumeropsis edulis*, chemical studies are not available in Gabon. The interest of this study is to determine the phytochemical composition and the antioxidant activity of these seeds and then to evaluate its therapeutic potential.

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Material and Methods:-

Plant Material

The fruits of *Cucumeropsis edulis* were collected in September 2018 at 50 km of Oyem city in northern Gabon. The shells containing seeds were dried at room temperature and under the sunlight. After extraction shells, the seeds were dried on oven at 90°C for 24 h. Mechanical crushing of the dried seeds produced a powder which 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 in Gabon.

Preparation of extracts

Extraction was performed using the protocol described by Koffi N'guessan (N'guessan et al., 2009) with few modifications. 150 mL of cyclohexane was added to 30 g of powder. The mixture was stirred during 24 h at room temperature. After filtration through Whatman No. 1 filter paper, the residue was dried on oven at 80°C for 24 h and the filtrate was concentrated in rotavapor to give oil with 43% yield. To the dried residue obtained, was added 150 mL of ethyl acetate and the mixture was stirred at room temperature for 24 h. After filtration through Whatman No. 1 filter paper, the residue was again dried on oven at 80°C for 24 h and the filtrate (**Ethyl acetate extract**) stored 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 was dried on oven at 80°C for 24 h and the filtrate (**Acetonic extract**) stored at 4°C until analysis. In finish, to the residual marcs, was added methanol. The mixture was stirred during 24 h at room temperature and filtrated through Whatman No. 1 filter paper allowed to the **Methanolic extract**.

The mixture of 30 g of dried powder of seeds and 150 mL of distilled water was heated in reflux for 1 h. After cooling at room temperature and filtration through Whatman No. 1 filter paper, the aqueous extract (Mefouet et al., 2021) was lyophilized and stored in the refrigerator at 4°C until evaluation of antioxidant activity.

Phytochemical analyses

Phytochemical screening is a qualitative analysis, based on staining or precipitation reactions. It permits identification of secondary metabolites. The analyses were released using standard methods with small modifications. The extracts were tested for the presence of **polyphenols, flavonoids, tannins, alkaloids, saponins, coumarins, reducing compounds, sterols and triterpenes**.

Polyphenols

1 mL Folin-ciocalteu reagent and 1mL sodium bicarbonate (Na_2CO_3) was added to 2 mL of filtrate. Dark-green coloration indicated the presence of polyphenols (Eyi et al., 2019).

Tannins

1 mL of 10% lead acetate solution was added to 3 mL of filtrate. White precipitate indicated the presence of tannins (Abogo et al., 2013). Differentiation of tannins was released with the mixture of 2 mL of filtrate, 2 mL of 1% copper sulfate solution and 2 drops of ammonia. Blue precipitate indicated the presence of gallic tannins and green precipitate catechic tannins (Adodo et al., 2015).

Alkaloids

A few drops of Dragendorff reagent were added to 2 mL of filtrate. A reddish-orange precipitate or coloration indicated the presence of alkaloids (N'guessan et al., 2009).

Flavonoids

1 mL of 1% ammonia solution was added to 4 mL of filtrate. Yellow coloration indicated the presence of flavonoids (Mahdjouba, 2015). For differentiate flavonoids, the mixture of 5 mL of filtrate, 5 mL of hydrochloric ethanolic solution, 1 mL of isoamyl alcohol and some magnesium chips was prepared. Appearance of orange pink coloration indicated the presence of flavones. Flavanones were detected by purplish pink coloration and red coloration indicated the presence of flavanols. The cyanidin reaction without magnesium and after heating for 10 minutes in the water bath showed a cherry-red color indicating the presence of leucoanthocyanins (Daira et al., 2016).

Sterols and triterpenoids

To 2 mL of filtrate were added a few drops of concentrated sulfuric acid. Observation of a purple coloration indicated the presence of triterpenes and green coloration for sterols (Daira et al., 2016).

Coumarins

3 mL of 10% sodium hydroxide solution was added to 2 mL of filtrate. After shaking the mixture, the appearance of a yellow color indicated the presence of coumarins (Daira et al., 2016).

Saponins

The mixture of 3 mL of distilled water and 2 mL of filtrate was shaken vigorously for 15 seconds. The observation of persistent foam for 20 minutes indicated the presence of saponins (Ano et al., 2018).

Reducing compounds

The mixture of 1 mL of Fehling's liqueur and 2 mL of filtrate was heated in a water bath for 15 minutes. The formation of a brick-red precipitate indicated the presence of reducing compounds (Ano et al., 2018).

Evaluation of the antioxidant activity

The antioxidant activity index (AAI) was determinate according to the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Scherer and Godoy (Scherer and Godoy, 2009; Nsi et al., 2019). Standard DPPH solution containing 10 mg of DPPH was prepared in 100 mL of ethanol. Different concentrations of extracts were prepared in ethanol ranging from 0.781 µg/mL to 100 µg/mL. 100 µL of standard DPPH solution was mixed with 100 µL of each extract solution. Absorbance was measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (vitamin C) was used as reference. Measures were released in triplicate. The scavenging activity of DPPH radical was calculated by using the following equation:

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

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 IC₅₀ which is the measure of concentration in µg/mL of extract that inhibits 50 % of DPPH radicals. The antioxidant activity index was calculated using the following formula:

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

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

Results and Discussions:-

The results of the phytochemical characterization of the crude extracts of the seeds of *Cucumeropsis edulis* are given in Table 1. These results show the presence of polyphenols, alkaloids, triterpenes and gallic tannins in all extracts. Leucoanthocyanins are present in the methanolic extract and reducing compounds in the ethyl acetate and acetonitrile extracts. Contrary to the results obtained in Benin (Yété et al., 2015), coumarins, catechic tannins and sterols are absent in all extracts like flavones, flavanones and flavanols. This difference could be explained by environmental, climatic and seasonal factors (Yété et al., 2015; Daddona et al., 1976). The absence of reducing compounds in methanolic extract shows that the quantity of these compounds is low in the seeds. All reducing compounds were then extracted by ethyl acetate and acetone. The absence of many groups of flavonoids suggests that polyphenols of this extract are in majority gallic tannins.

The abundance of secondary metabolite like polyphenols, alkaloids and gallic tannins can give many biological properties to the seeds of *Cucumeropsis edulis*. Polyphenols have shown to be antioxidants, anti-inflammatories and anti-hypertensives (Yété et al., 2015; Amiot-Carlin, 2014; Léotoing et al., 2014). Alkaloids have analgesic, anti-diabetic, anti-cancer, anti-microbial, anti-tumor and anti-parasitic effects (Yété et al., 2015; Mangambu et al., 2014; Kabore et al., 1997; Agostinho, 2013). Tannins are antibacterials, antioxidants, anti-inflammatories, anti-diabetics (Mangambu et al., 2014; Adaramoye et al., 2005; Yété et al., 2014). Triterpenes can fight inflammation (Yété et al., 2015). Finally, leucoanthocyanins have a diuretic (antihypertensive) action (Yété et al., 2015). The presence of these phytochemicals in the seeds of *Cucumeropsis edulis* extracts could prevent risks against pathologies such as cardiovascular diseases, hypertension, diabetes and cancer.

Table 1:- Results of phytochemical screening.

Phytochemicals	Ethyl acetate extract	Acetonic extract	Methanolic extract
Polyphenols	+	++	++
Gallic tannins	+	++	++
Catechic tannins	-	-	-
Alkaloids	+	++	++
Flavones	-	-	-
Flavanone	-	-	-
Flavanols	-	-	-
Leucoanthocyan	-	-	++
Reducing Compounds	++	+	-
Saponins	-	-	-
Sterols	-	-	-
Triterpenes	++	++	+
Coumarins	-	-	-

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

The results of the evaluation of the antioxidant activity by the anti-free radical activity index (IAA) method are given in Table 2. The index of the anti-free radical activity is 0.022 ± 0.001 . According to this index, the antioxidant activity can be considered low ($IAA < 0.5$), moderate ($0.5 < IAA < 1$), high ($1 < IAA < 2$) and very high ($IAA > 2$) (Scherer and Godoy, 2009). The antioxidant activity of seeds of *Cucumeropsis edulis* is then low despite the abundance of polyphenols. These results agree with those described in the literature (Yété et al., 2015). The low antioxidant activity is in according to the phytochemical screening through the absence of many groups of flavonoids. Similarly, the low antioxidant activity could be explained by the absence of condensed tannins (Ba et al., 2010). indeed, catechic tannins have a stronger antioxidant activity than gallic tannins.

Table 2:- Results of the evaluation of antioxidant activity.

	Equation	R ²	IC ₅₀ (μ g/mL)	IAA
Plant material	$Y = 0,021X - 0,2$	0,882	$2249,42 \pm 87,03$	$0,022 \pm 0,001$
Ascorbic acid (standard)	$Y = 5,120X + 01,46$	0,971	$4,89 \pm 2,8$	$10,22 \pm 0,9$

Conclusion:-

The phytochemical characterization of the extracts of *Cucumeropsis edulis* seeds identified polyphenols, alkaloids, gallic tannins, reducing compounds, triterpenes and leucoanthocyanins. These compounds have known to have many biological properties. However, saponins, catechic tannins, sterols, coumarins, flavones, flavanones and flavanols are absent. These results suggest that the polyphenols of this seeds are in majority gallic tannins. Despite the low antioxidant activity attributed to the absence of many groups of flavonoids and catechic tannins, the use of seeds of *Cucumeropsis edulis* in the diet or herbal medicine could protect populations against many diseases.

References:-

1. Abogo Mebale, A.J., Suebang, F.C., Ondo Azi, A.S., Yemelong, J.N., Ondo, J.A., NsiEmvo, E., and Eba, F. (2013): Chemical composition of a standard sugarcane wine of *Saccharum officinarum* Linn from Woleu-Ntem, Gabon. *Journal of Agriculture and Sustainability*, 3(2): 216-222.
2. Adaramoye, O.A., Farombi, E.O., Adeyemi, E.O., and Emerole, G.O. (2005): Comparative study of the antioxidant properties of flavonoids of *Garcinia kola* seeds. *Pak. J. Med. Sci*, 21: 142-149.
3. Adodo, K., Elolo, O., Bilabina, I., Ayélé, C.T., Yao, B., and Courdjo, L. (2015) : Caractéristiques chimiques et physicochimiques des grains de cucurbitacées du Togo : concombre amer (*Cucumeropsis edulis* hook.f.) et melon à pistache (*Citrullus lanatus* Var). *Journal de la société Ouest-Africaine de chimie*, 40 :1-7.
4. Agostinho, D. (2013) : Investigation phytochimique de plantes utilisées en médecine traditionnelle au Mozambique : *Ptaeroxylon obliquum* Radlk, *Pyrenacanthakaurabassana* Baill, *Monadenium lugardiae* N.E.Br. M. Sc. Thèse, Université François-Rabelais de Tours, France.

5. Amiot-Carlin, M.J.(2014) : Les phytomicronutriments : tour d'horizon et difficultés rencontrées pour établir des ANC. Liens avec la réglementation des allégations de santé. *Innovations agronomiques*, 42 : 1-9.
6. Ano, A.A.R.R., Koffi, E.N., Adima, A.A., N'da, P.H., and Anin L.A. (2018): Composition biochimique et phytochimique des tourteaux des fruits du safoutier (*Dacryodes edulis*) de Côte d'Ivoire. *Int. J. Biol. Chem. Sci.*, 12(6): 2535-2546.
7. Ba K, Tine E, Destain J, Cissé N, and Thonart P.(2010) : Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt. *Biotechnol. Agron. Soc. Environ.*, 14(1) :131-139
8. Daddona, P.E., wright, J.L., and Hutchinson, C.R. (1976): Alkaloid catabolism and mobilization in *Catharanthus roseus*. *Phytochem*, p 941-945.
9. Daira, N.E.H., Maazi, M.C., and Chefrou, A.(2016) : Contribution à l'étude phytochimique d'une plante médicinale (*Ammoides verticillata* Desf. Briq.) de l'Est Algérien. *Bulletin de la Société Royale des Sciences de Liège*, 85 : 276 – 290.
10. Eyi Mints, B.A., Ibrahim, Mabika Mabika, R., MveMendame, W.L., Lepengue, A.N., Souzal, A., et coll.(2019) : Etude phytochimique et activités biologiques de deux plantes: *Alchornea floribunda* (euphorbiaceae) et *Bridelia ferruginea* (phyllanthaceae). *International journal of advanced research*, 7(3): 331-345.
11. Kabore, Z.I., and Millogo, K.H.(1997) : Etude antibactérienne in vitro d'extraits alcaloïdiques de *Holarrhena floribunda*, vis-à-vis de *Escherichia coli* enteropathogène, sérotype O₁₂₇. *Pharm. Méd. Trad. Afr.*, 9: 17-23.
12. Léotoing, L., Darie, C., Guicheux, J., Miot-Noirault, E., Coxam, V., and Wittran, Y. (2014) : L'innovation nutritionnelle au service de la prévention de l'ostéoporose. Cas de la fisétine : un polyphénol capable de protéger notre capital osseux. *Innovations agronomiques*, 42: 39-46.
13. Mahdjouba, H.(2015) : Etude phytochimique de *Rosmarinus officinalis* (L) et *Marrubium vulgare* (L) (Aspect qualitatif). Master, Université Ziane Achour de Djelfa, Algérie.
14. Mangambu Mokoso, J.D., Mushagalusa Kasali, F., and Kadima Ntokamunda, J. Contribution à l'étude phytochimique de quelques plantes médicinales antidiabétiques de Bukavu, R.D Congo. *Journal of Applied Biosciences*, 2014; 75 :6211– 6220.
15. Mefouet Abessolo, D.D., Abogo Mebale, A.J., Allogho Mve, J., and Ondo, J.A. (2021): Phytochemical characterization and of total polyphenols and flavonoids content of the aqueous extract of the seeds of *Cucumeropsis edulis* (Cucurbitaceae) from Gabon. *Journal of Pharmacognosy and Phytochemistry*, 10(2):138-141.
16. Narenda, V., Mukul, T., Narayan Prasad, G., and Bhaskar, K.G. (2010). Antioxidant potential of *Psidium guajava* Linn. *International Journal of PharmTech Research* 2(1):417-419.
17. Ndola, B.P.(2015). Etat des lieux de la filière apicole en République Démocratique du Congo et évaluation des capacités pollinisatrices des abeilles (*Apis mellifera adansonii* L.) sur la culture de melon africain (*Cucumeropsis mannii*, Naudin) à Kisangani. Thèse doctorat, Université de Liège Gembloux Agro-biotech, Liège, Belgique.
18. N'guessan, K., Kadja, B., Zirihi, G.N., Traoré, D., Aké-Assi, L., (2009) : Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Abboville, Côte-d'Ivoire). *Sci. Nat.*, 6(1): 1-15.
19. Nsie Akoue, G., Mbehang Nguema, P.P., Onanga, R., Mabika Mabika, A., and Ibrahim, B. (2019): Nutritional prospects, antioxidant and antibacterial activities of some food plants consumed by wild mandrill (*Mandrillus sphinx*) population from Lékédi park (BAKOUMBA, GABON). *Int. J. Adv. Res.*, 2019; 7(2): 116-136.
20. Pl@nt use. (accès 2020) *Cucumeropsis mannii* (PROTA). Disponible auprès de [http://www.uses.plantnetproject.org/fr/Cucumeropsis_mannii_\(PROTA\)](http://www.uses.plantnetproject.org/fr/Cucumeropsis_mannii_(PROTA)).
21. Raponda, W.A., and Sillans, R.(1995) : Les plantes utiles du Gabon. Edn. Sépia.
22. Savadogo, A., Zongo, C., Bayala, B., Somda, K.M., and Traoré, A.S. (2011). Nutritional potentials of *Cucumeropsis edulis* (Hook. f.) Cogn seeds and the pulp of *Adansonia digitata* L. from Burkina Faso: Determination of chemical composition and functional properties. *International Food Research Journal*, 18(4): 1409-1414.
23. Scherer, R., and Godoy, H.T. (2009): Antioxidant activity index (AAI) by 2, 2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, 112: 654-658.
24. Yété, P., Togbé, A., Koudoro, Y., Agbangnan, P., Ndahischimiye, V., Djènantin, T.S., Wotto, D., Azandégbé, E.C., Sohounhloué, D. (2015): Comparative study of phenolic compounds and radical-scavenging activity of the extracts of seeds of *Garcinia kola* (Guttiferae) and *Cucumeropsis edulis* (Cucurbitaceae) of Benin. *International Journal of Innovation and Scientific Research*, 15(1): 217-227.
25. Yété, P., Ndayishimiye, V., Agbangnan, P.C., Djènantin, S.T., Wotto, V.D., and Sohounhloué, D.C.K. (2014): Chemical Composition of the Seeds and the Defatted Meal of *Garcinia kola* Heckel (Guttiferae) from Benin. *Chemistry Journal*, 4(5):13-19.