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## Research Article

### ANTIOXIDANT CAPACITY AND MINERAL CONTENTS OF FIVE SPECIES OF CUCURBITACEAE SEEDS FROM CAMEROON

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#### ABSTRACT

Cucurbitaceae seeds are widely used to prepare African sauces. Their proximate analysis has shown their nutritional potential as sources of macronutrients for humans. They are also used for medicinal purposes but their contents in phytochemicals and minerals that could explain their utilization are not yet investigated in Cameroonian context. This paper therefore analyses some secondary metabolites, the total antioxidant and antiradical abilities of oils, phenol and mineral contents in the seeds of *Lagenaria siceraria*, *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata* and *Cucumis sativus*, from different agro-ecological zones in Cameroon. The methods of screening of phytochemicals and antioxidant evaluation have been described in detail. The mineral content was by flame absorption spectrophotometry on ash solutions except for phosphorus which was by colorimetry. The results showed that saponins, tannins, flavonoids, alkaloids, phenols and triterpenes were present in all the seeds, except *C. mannii* which had no saponins. The phenol content varied from 299 (*C. mannii*) to 735mg Gallic Acid Equivalent (EAG) /100g dw (*C. maxima*). These secondary metabolites confer to them antimicrobial properties. The total antioxidant capacity ranged from 50 (*C. moschata*) to 97.78 mgEq.catechine /g dw (*C. sativus*). *L. siceraria* oil had the highest antiradical activity (0.083). The high antioxidant capacities of these seeds (especially *C. sativus*) and the abilities of their oils to trap the DPPH• radical (especially *L. siceraria* oil which was similar to that of Olive oil), suggest their potential use against cardiovascular diseases and prostate cancer. These seeds were rich in minerals, especially *C. moschata* with the highest levels of phosphorus (810.26), potassium (681.38), magnesium (586.21), sodium (2.01), iron (5.06), zinc (5.46) and manganese (2.81 mg /100g dw). The Na/K<1 and high Zn levels suggest their potential use against hypertension and enhancement of the immune system for people living with HIV/AIDS.

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#### INTRODUCTION

Cucurbitaceae seeds are part of the major ingredients used in the preparation of African sauces that complement starchy foods. When they are washed and dried and decorticated, they can be soaked in water for milk preparation (Kinkela, 1990), ground into a paste for the thickening of sauces or prepared into a thick delicious paste that is wrapped in plantain leaves (known as *egusi pudding*) and used as sauce for eating starchy foods. They can be used for the extraction of edible oil (Fokou *et al.*, 2009). Many uses of these seeds for medicinal purposes have been described. The fresh seeds are eaten to relieve abdominal pains and distensions caused by intestinal worms (Rahman *et al.*, 2008). Oils extracted from these seeds have beneficial effects on lipid profile such as increasing HDL-cholesterol levels and decreasing LDL-cholesterol levels (Achu *et al.*, 2008), whose oxidation is at the origin of atherosclerosis. Oils from some Cucurbitaceae seeds are used for the treatment of benign prostatic hyperplasia (Gossell-Williams *et al.*, 2006).

The proximate composition of Cucurbitaceae seeds have shown their potential as source of macronutrients for humans, for their protein and lipid levels are from 24-41.6 % and 42 – 57.3% respectively (Fokou *et al.*, 2004). Although these macronutrients contribute to the health benefits of these seeds through the quality of their amino acids and fatty acids, a knowledge of phytochemical compounds (such as phenolic compounds which are secondary metabolites of plant metabolism) and minerals could further justify the medicinal properties of these Cucurbitaceae seeds. Bruneton (1999) stated that phenolic compounds are responsible for numerous physiological actions in the organism due to their anti-carcinogenic, anti-inflammatory, anti-atherosclerosis and anti-tumoral activities; flavonoids are veinotonic (ease blood circulation); terpenes have bactericidal, antiulcerative, antiviral and antifungal effects and saponins have antihelminthic properties. Phytochemical compounds ensure the protection of the organism against degenerative diseases such as cancer and atherosclerosis, diseases caused by reactive oxygen species

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(hydroxyl and superoxide radicals, hydrogen peroxide) (Dykes and Rooney, 2007). Minerals such as trace elements (copper, zinc, manganese and selenium) are necessary for the activity of antioxidant enzymes. They associate with antioxidants (phenolic compounds, alkaloids, terpenes, carotenoids) to constitute the first line of defense against reactive oxygen species in the organism (Lehucher-Michel *et al.*, 2001; koechlin-Ramonatxo, 2006).

The health benefits of Cucurbitaceae seeds can only be effective if they are consumed at the amounts that can bring these phytochemicals and minerals in active concentrations. To the best of our knowledge, there no data on the phytochemical and mineral contents of Cucurbitaceae seeds currently consumed in Cameroon. This research is therefore aimed at studying the phytochemical compound profile, antioxidant activity and some mineral contents of the seeds of *Lagenaria siceraria*, *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata* and *Cucumis sativus*, which are the most widely consumed Cucurbitaceae seeds in Cameroon. The results could contribute to the management of micronutrient deficiency which affects about two billion people in the World (UNICEF / MI / GAIN / USAID, 2009) and which is a public health concern in Cameroon.

## MATERIALS AND METHODS

### Collection of Samples and regions of collection

Cucurbitaceae are annual herbaceous and monoecious plants, made up of about 118 genera and 825 species. They have long flexible climbing stems with spirals. Their fruits have variable colours and shapes with a waterproof skin protecting the pulp containing numerous seeds (Gemrot *et al.*, 2006; Mladenovic *et al.*, 2008).

The Cucurbitaceae seeds were collected already dried under local conditions by the farmers. Collection was done from May to July, 2010 from 3 agro-ecological zones in Cameroon which are the high Plateaus of the West, the Humid and Swamp Forests (Table 1), for it has been shown that the region of cultivation has an influence on the chemical composition of the seeds.

selecta) at 70°C for 24 h and ground with an electrical grinder. The powder was defatted and the oil was obtained by cold extraction according to the method of Folch and Sloane (1957), modified by Xanthopoulou *et al.* (2009). 10 g of sample were weighed into a beaker and 50 ml of dichloromethane/methanol mixture (2/1, v/v) added. The mixture was stirred with a magnetic stirrer for 15-20 min and filtered with filter paper. 50 ml of solvent were added into the residue and the operation repeated. 20 ml of NaCl 0.9 % were added to the combined liquid phases. The filtrate was put into a separatory funnel and vigorously shaken. The funnel was mounted to a support and allowed to stand for 24h at room temperature for separation of the two phases. The lower phase containing dichloromethane-dissolved lipids was collected into a beaker, covered with a thin cloth and left at room temperature for 24h for the solvent to evaporate. The oil obtained was used for the assay of antiradical activity.

Hot extraction of the oil was also done according to AOAC (1980). 10g of sample were submitted under continuous reflux with hexane at 68°C in a Soxhlet apparatus. The defatted cake obtained after 8h of oil extraction was used for the determination of the mineral content. Another portion of the defatted cake obtained after 2h of extraction was used for phytochemical screening and for the preparation of hydro-ethanol extracts (for the evaluation of total phenol content) and also for the preparation of hydro-methanol acid extracts (for the evaluation of total antioxidant capacity).

### Phytochemical screening

#### Preparation of extracts

10 g of sample were defatted for 2 h in a Soxhlet extractor. This was to eliminate chlorophyll pigments that could interfere with the appreciation of colour indicating the presence of desired compounds during phytochemical screening and to minimize interferences that could be caused by oil during the quantification of phenols, evaluation of the total antioxidant capacity (Arranz *et al.*, 2008; Olayinka and Okoh, 2010).

2 g of defatted sample were boiled in 20 ml of distilled water for one hour.

**Table 1** Regions of collection of samples

Sample	Bioclimatic zone	Region	Locality
<i>Lagenaria siceraria</i>	High plateau of the West	North West	1- Mankon, 2- Ndop, 3- Wum
		West	4- Bafoussam, 5- Dschang, 6- Bamougoum
<i>Cucumeropsis mannii</i>	High plateau of the West	North West	7- Bangolan, 8- Wum, 9- Essimbi
		West	10- Baloum, 11- Bamougoum, 12- Galim
<i>Cucurbita Maxima</i>	High plateau of the West	North West	13- Weh, 14- Kom, 15- Bangolan
		West	16- Galim, 17- Bangangté, 18- Foubot
<i>Cucurbita moschata</i>	High plateau of the West	North West	19- Mankon, 20- Zhoa, 21- Wum
		West	22- Dschang, 23- Foubot, 24- Bamougoum
<i>Cucumis Sativus</i>	Humid Forest	Centre	25- Bafia, 26- Mbalmayo, 27- Obala
<i>Olive oil</i>	Swamp Forest	South West	28- Buea, 29- Mamfé, 30- Mundemba 31- Olive oil

\*The number given to each locality represents the code of the sample collected

### Treatment of Samples

The collected seeds were decorticated, sorted to discard unhealthy seeds and dirt, dried in an air-ventilated oven (P-

The mixture was filtered on a Buchner funnel and the filtrate was used to assay for tannins and saponins. Another 2 g of defatted sample were soaked in 20 ml of water-ethanol solvent

(30:70 v / v). After 48 h, the mixture was filtered and the filtrate was used for the analysis of phenols, flavonoids and alkaloids. The screening was done according to Harbone (1976).

**Test for saponins:** 5 ml of the filtrate were added to 2.5 ml of distilled water in a test tube. The mixture was vigorously stirred on a vortex mixer for one minute. The appearance of persistent foams was indicative of the presence of saponins.

**Test for tannins:** A few drops of 3% ferric chloride solution were put in 5 ml of filtrate. The formation of a dark brown, greenish brown or blue-green precipitate was indicative of the presence of tannins.

**Test for flavonoids:** A few magnesium pellets were added to 3 ml of hydro-ethanol extract. Some drops of concentrated HCl were added. The appearance of a brick red or violet effervescence indicated the presence of flavonoids.

**Test for Alkaloids:** 1 ml of 1% HCl was mixed with 3 ml of hydro-ethanol extract in a test tube. A few drops of Meyer reagent were added. The appearance of a cream white precipitate indicated the presence of alkaloids.

**Test for phenols:** 5 ml of hydro-ethanol extract were added to 3 drops of freshly prepared ferric cyanide. The formation of a green precipitate marked the presence of phenols while a blue precipitate indicated the presence of polyphenols.

**Test for triterpenes:** 50 mg of sample powder were mixed with 2 ml of chloroform. 4 drops of acetic anhydride and concentrated H<sub>2</sub>SO<sub>4</sub> were added. The presence of triterpenes was indicated by the appearance of a purplish red colour.

#### Total phenol Content

The total phenol content was evaluated by spectrophotometry as described by Olayinka and Okoh (2010). 200 µl of extract were put into a test tube and 2 ml of 10 % (v/v) Folin-Ciocalteu reagent were added, stirred and allowed to stand for 5 min. 2 ml of 10% sodium carbonate were added, vigorously stirred with a vortex mixer and allowed to stand again for 30 min at room temperature (25 ± 1°C). The absorbance was read at 765 nm against the blank. Gallic acid (0-0.25 mg/ml) was used to construct a standard curve for quantification of phenolic content of the samples and the results were expressed as mg gallic acid equivalent (GAE)/100 g dw

#### Total antioxidant capacity

The FRAP (Ferric Reducing Antioxidant Power) method was used for the determination of total antioxidant capacity, according to Benzie and Strain, (1996). This method measures the reducing power of the antioxidant by transfer of electrons. FRAP measures the ability of the sample to reduce iron to a low pH (3.6). Yellow tripyridyltriazine ferric complex (Fe<sup>3+</sup>-TPTZ) is reduced to blue ferrous tripyridyltriazine complex (Fe<sup>2+</sup>-TPTZ) by an electron donating substance under acidic conditions (pH 3.6). An intense colour blue is formed when ferrous tripyridyltriazine complex is formed. The absorbance is measured at 593 nm.

The FRAP reagent was composed of, 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2,4,6 - tris (2-pyridyl)-s-triazine) in 400 mM HCl, 10mM Ferric chloride (FeCl<sub>3</sub>) in the ratio: 10 : 1 : 1, v/v/v.

The acidic hydro-methanol extract was prepared as described by Agbor (2005). 100mg of defatted sample were put into a test tube and 10 ml of HCl 1.2N in methanol 90% were added. The tubes were tightly corked and placed in a Water bath at 90°C for 90 min, while shaking every 30 min. After cooling, the tubes were centrifuged at 3500g, the supernatant collected, its volume made to 10 ml and labeled. 75 µl of this extract were put into a test tube and 2 ml of FRAP reagent added. The mixture was stirred, incubated for 6 min at room temperature and the absorbance read at 593nm, using a Spectrophotometer (JENWAY 6315). The antioxidant capacity of each sample expressed as mg catechin equivalent/g dry weight was obtained by projecting its absorbance on the standard curve. The tests were performed in duplicate.

#### Antiradical activity of oils

The determination of the antioxidant activity by the DPPH-antiradical method is based on the decrease in absorbance at 515nm due to a change in colour from violet (DPPH<sup>•</sup>) to yellow (DPPH) as the radical gets trapped by antioxidants, through the transfer of a hydrogen atom from the antiradical to give stable DPPH-H. The ability of Cucurbit oils to trap the DPPH<sup>•</sup> radical (1,1-Diphenyl-2-picirilhydrazyl) was evaluated as described by Sanchez *et al.* (1998). 3.9 ml of DPPH (60 µM) prepared in ethyl acetate was added to 0.1 ml of Cucurbit oil at 2%, 1%, 0.5%, 0.25% and 0.125% in ethyl acetate. The mixture was vigorously stirred and incubated in the dark for 30 min. The same procedure was used for olive oil, in order to compare the activity of Cucurbit oils to that of oil with high antioxidant capacity. A control solution was prepared made up of DPPH solution and 0.1 ml ethyl acetate. The absorbance was read at 515 nm and the percentage of inhibition of DPPH radical was calculated as follows:

$$\% \text{ d'in ibration} = \frac{Abs(0) - Abs(t)}{Abs(0)} \times 100$$

Abs (0) = Absorbance of control at t = 0 min  
Abs (t) = Absorbance of the extract after incubation

The tests were performed in duplicate and the parameters EC<sub>50</sub> (effective concentration 50) and AP (antiradical power) determined. The EC<sub>50</sub> (the concentration of the antiradical that is able to trap 50% of free radicals) was obtained from the graph of the percentage of trapping (inhibition) versus the concentration of sample. This value depends on the concentration (mg/ml) of DPPH<sup>•</sup> used for the test.

The antiradical power (AP) is the inverse of EC<sub>50</sub>. It measures the effectiveness of the antiradical. The higher the AP, the more effective the antiradical. AP was determined as:

$$AP = 1/EC_{50}$$

#### The Mineral content

The mineral contents were assayed by atomic absorption spectrophotometry as described by Benton and Vernon (1990). This method consists of vapourising a liquid sample and heating it with a flame. The flame is directed towards light from a lamp that emits wavelengths which are characteristic of the desired mineral. On crossing the flame, light waves whose wavelengths correspond to the assayed mineral are absorbed by

excited ions present in the flame. The absorbance measured is directly proportional to the concentration of the mineral.

A porcelain capsule was washed, rinsed with 10% nitric acid, dried in an oven for 30 min and in a furnace for 3 hours. 0.5g of defatted sample were put into the capsule. An empty capsule served as the blank. The capsules were placed in the furnace at 500°C for 24h, then cooled in a desiccator. A whitish ash was obtained. The capsules were rinsed with 15 ml of aqua regia solution (400ml of concentrated HCl, 133 ml of 70% nitric acid, and 1.467 l of deionized water) and put into 50 ml propylene tubes. The mixture was stirred for 10 min on a magnetic stirrer and centrifuged at 3000 turns/min for 10 min. The supernatant (the sample solution) was collected for analyses. For the assay of macrominerals (Ca, Mg, K, Na), 0.5 ml of supernatant was diluted in 19.5 ml of strontium chloride solution (5.75 g of SrCl<sub>2</sub>, 6H<sub>2</sub>O was dissolved and made to 2l with deionised water). For the assay of trace elements (Cu, Fe, Mn, Zn), about 10 ml of undiluted supernatant were used. 2 tubes with the same quantities of reagents as the others and made to the mark with deionised water were added to each set of analyses. The solutions obtained (standards, samples and blanks) were passed through a flame atomic absorption spectrophotometer and the contents in calcium, magnesium, potassium, sodium, iron, zinc, copper and manganese were determined at 422.7, 285.2, 766.5, 589, 248.3, 213.9, 324.7, 279.5 nm respectively. A standard curve for each standard solution enabled the determination of the content (mg/100g dw) of each mineral, using the equation of the straight line.

The phosphorus content was determined as described by Murphy and Riley (1962). In acid and reducing media, phosphate ions react with ammonium molybdate to give a blue phospho- ammonium molybdate complex whose absorbance at 860nm is proportional to the concentration of phosphorus. 0.25 ml of each sample solution were put into 25ml tubes and 19.75 ml of the working solution (1.056 g of ascorbic acid dissolved in 1L of deionised water and 200 ml of Murphy-Riley stock solution) were added.

The solutions (standard, samples and blanks) were allowed to stand for 30 min for colour to develop and the absorbance was read at 860 nm against the blank with a spectrophotometer. The analyses were done in duplicate. The phosphorus content of each sample was obtained by using the equation of the standard curve.

### Statistical Analyses

The results presented as mean  $\pm$  standard deviation, were analyzed by Analysis of Variance (ANOVA) and LSD (Least Significant Difference) at 5% level of significance using the software Stagraphics plus 5.0 for Windows. The statistical package, SPSS 12.0, using Friedman's test, enabled us to classify the Cucurbit seeds according to their mineral contents.

## RESULTS AND DISCUSSION

### Phytochemical Screening

The phytochemical analysis of the Cucurbitaceae seeds showed that they all contain tannins, flavonoids, alkaloids, phenols and triterpenes (Table 2). *C. sativus* had the highest level of tannins. These seeds also contain saponins except *C. mannii*. A

characterization of the different classes of secondary metabolites of *Citrullus lanatus* var. *citroides* by Hassan *et al.* (2011) also revealed the presence of flavonoids and terpenes. Saponins, phenols and tannins were averagely present while alkaloids were absent. In general, the phytochemical compounds did not depend on the region of cultivation of the seeds but on the specie.

Saponins are secondary metabolites with antibacterial and antihelminthic activities. Tannins have anti-inflammatory properties. The presence of saponins, tannins, flavonoids, alkaloids and terpenes could be responsible for the antimicrobial properties of these seeds, which explains their use in traditional medicine for the treatment of gastrointestinal infections. In a study on the anthelmintic efficacy of pumpkin seed (*Cucurbita pepo* Linnaeus, 1753) on ostriches naturally infected with gastrointestinal nematodes in Paraíba State, Brazil, it was shown that ostriches that received 1g/kg body weight of pumpkin seed meal (treated orally for 3 consecutive days, at intervals of 7 days, totaling 9 administrations), showed a significant decrease in egg counts per gram of faeces, while the control group (received no treatment) and drug group (treated once, with Albendazole 5%, at the beginning of the experiment according to the manufacturer's recommendations) showed no reduction in egg counts per gram of faeces. This showed that the administration of pumpkin seed was effective in controlling gastrointestinal helminths in naturally infected ostriches (Feitosa *et al.*, 2013). Hence *C. pepo* also known as *C. moschata* has potential for the fight against intestinal nematodes.

### The Total Phenolic Content

The total phenolic content of the Cucurbit seeds varies according to the specie, from 299 (*C. mannii*) to 735mgEAG/100g (*C. maxima*) (Table 3). For *L. siceraria*, *C. moschata* and *C. sativus*, the phenolic content depends on the region of cultivation.

The values obtained for *C. maxima*, *C. moschata* and *C. sativus* seeds, are higher than those obtained by Achu *et al.* (2013) which are 420 (*C. maxima*, *C. moschata*) and 430mg/100g (*C. sativus*), while those for *C. mannii* and *L. siceraria* are closer to the values obtained by these Authors which are 390 and 340 mg/100g respectively. These values are higher than those reported by Kubola and Siriamornpun (2011) for seeds of *Momordica cochinchinensis* (201 mgEAG/100g MS).

Phenolic compounds are the major contributors to the antioxidant activity in plants. These molecules which are natural antioxidants can inhibit the oxidation of low density lipoprotein (LDL), the primary cause of atherosclerosis. They therefore play a protective role against cardiovascular diseases (Halliwell, 2007). On the other hand, these compounds (tannins) can also bind to proteins and carbohydrates thereby reducing their bioavailability. They are also chelators of minerals. Phytate for example can form complexes with divalent cations such as calcium, zinc and iron, reducing their bioavailability (Aberoumand, 2011). It is therefore important to identify and determine the content in the different types of phenolic compounds in the seeds, in order to appreciate their nutritional importance.

### The Total antioxidant capacity

The total antioxidant capacity of the Cucurbit seeds are found on Table 4. The values range from 50 (*C. moschata*) to 97.78 mgEq.catéchine/g dw (*C. sativus*). They depend on the specie. These values are higher than those reported by Agbor (2005) for *C. mannii* seeds (39.23 mgEq.catechine /g dw).

Polyphenolic compounds can be present in free, soluble or insoluble, esterified/etherified forms, or bound to cell wall components such as polysaccharides, proteins, lignin, cutin or suberin. Insoluble polyphenols are considered as the major contributors to the total antioxidant capacity (Serpen *et al.*, 2008). Acid hydrolysis breaks the cell walls, liberating bound polyphenols (Arranz and Calixto, 2010).

The acidic hydro-methanol extracts of these seeds, which can reduce iron, (a catalyst for lipid oxidation and the genesis of the hydroxyl radical (HO<sup>•</sup>) via Fenton and Haber-Weiss reactions) could therefore help to reduce the formation of free radicals and dislocation of cell membranes, hence reducing cell proliferation and tumor formation.

test shows that all the oils trap the radical with efficiency depending on the specie and not on the agro-ecological zone of cultivation (Table 4). *L. siceraria* seed oil has the highest antiradical power (0.083 ml / mg), while *C. moschata* and *C. maxima* seed oils have the lowest (0.030 and 0.034 ml / mg respectively). Olive oil, whose capacity to trap the DPPH• radical was also evaluated, had a higher antiradical power (0.095 ml/mg) than oils from *C. mannii*, *C. moschata*, *C. maxima* and *C. sativus*. There is no significant difference between the value obtained for *L. siceraria* oil and that of olive oil. The ability of these oils to trap the DPPH • radical shows that they contain compounds that can donate a hydrogen atom to form stable DPPHH.

**Table 2** Phytochemical Screening

Specie	Agro-ecological zone	Regions	Code	Saponins	Tannins	Flavonoids	Alcaloids	Phenols	Triterpenes	
<i>L. siceraria</i>	High Plateau of the West	North	1	+	+	+	++	++	++	
			2	+	++	+	++	++	++	
		West	3	++	++	++	+++	++	++	++
			4	++	++	+	+	++	++	++
			5	+	+	+	++	++	++	++
			6	+	+	++	++	+	++	++
<i>C. mannii</i>	High Plateau of the West	North	7	-	+	++	++	++	++	
			8	-	+	+++	+++	++	+++	
		West	9	-	+	+	+	++	++	+
			10	-	+	+++	++	++	++	+
			11	-	+	+	++	++	++	++
			12	-	+	++	++	++	++	++
<i>C. maxima</i>	High Plateau of the West	North	13	+	+	+	++	++	++	
			14	+	+	+	+++	++	++	
		West	15	+	++	+	++	+	++	++
			16	++	+	+	++	++	++	+++
			17	+	+	++	++	++	++	++
			18	++	+	+	+	++	++	+
<i>C. moschata</i>	High Plateau of the West	North West	19	+	+	+	++	++	+	
			20	++	+	++	+	++	++	
		West	21	++	+	++	+++	++	++	+++
			22	++	+	+	++	++	++	+++
			23	++	+	++	+	++	++	+++
			24	+	+	+	+	++	++	++
<i>C. sativus</i>	Humid Forest	Centre	25	+	+++	+	+++	+	++	
			26	+	+++	+	++	++	+++	
	Swampy Forest	South West	27	+	+++	+	++	++	++	
			28	+++	+++	++	+	++	+++	
			29	++	+++	+	++	++	+++	
			30	+	+++	++	++	++	++	

**Table 3** Total Phenol Content

Specie	Agroecological Zone	Region	Phenol content (mgEAG/100gMS)
<i>L. siceraria</i>	High Plateau of the West	North West	471.39 ± 6.16
		West	209.25 ± 3.72
		<b>Average ± SD</b>	<b>340.32 ± 2.93<sup>a</sup></b>
<i>C. mannii</i>	High Plateau of the West	North West	314.75 ± 21.39
		West	283.50 ± 14.48
		<b>Average ± SD</b>	<b>299.13 ± 10.54<sup>a</sup></b>
<i>C. maxima</i>	High Plateau of the West	North West	722.58 ± 107.65
		West	747.42 ± 99.62
		<b>Average ± SD</b>	<b>735.00 ± 59.88<sup>b</sup></b>
<i>C. moschata</i>	High Plateau of the West	North West	970.77 ± 120.07
		West	298.33 ± 59.70
		<b>Average ± SD</b>	<b>634.55 ± 54.75<sup>b</sup></b>
<i>C. sativus</i>	Humid Forest	Centre	705.54 ± 67.56
	Swampy Forest	South West	477.63 ± 54.18
		<b>Average ± SD</b>	<b>591.59 ± 35.35<sup>b</sup></b>

**Fisher Test:** Values with the same letter superscript within a column are not statistically different (p <0.05).

**The Antiradical activity**

The evaluation of the antiradical activity of the oils extracted from these Cucurbit seeds, by the DPPH• radical scavenging

Vegetable oils contain antioxidants such as tocopherols, carotenes and small quantities of polar bioactive compounds (Fruhwrith *et al.* 2003). *L. siceraria*, *C. sativus*, *C. mannii*, *C.*

*maxima* and *C. moschata* seed oils are rich in polyunsaturated fatty acids especially linoleic acid (69.1, 61.6, 55.2, 52.4 and 49.5% respectively). They also contain palmitic, stearic, oleic, arachidonic and linolenic acids in varying amounts (Fokou *et al.*, 2009). The high antiradical capacity of some of these oils (*L. siceraria*) could be due to the difference in composition and structural characteristics of polar bioactive and unsaponifiable compounds in these oils.

Considering their antioxidant capacity and the effects of *C. mannii* and *C. sativus* on lipid profile such as the lowering effect on LDL cholesterol and increasing HDL-cholesterol levels (Achu *et al.*, 2008), these oils are to be considered in the prevention and fight against cardiovascular diseases. Their high antiradical activity could explain their use in the treatment of urinary disorders (cystitis) and benign prostatic hyperplasia (Gossell-Williams *et al.*, 2006).

### Macrominerals

From the macrominerals, phosphorus, magnesium and potassium predominate, followed by Calcium and sodium which is the least abundant (Table 6). The **calcium** content varies from 48 (*C. maxima*) to 90.06 mg/100g dw (*C. sativus*).

These values are lower than those reported by Alfawaz (2004) for *C. maxima* seeds (139.70 mg/100g dw) but higher than that of *L. siceraria* seeds (40 mg/100g dw) by Olafoe *et al.* (2009). *C. moschata* seeds have the highest **phosphorus** content (810mg) while *C. sativus* seeds have the lowest (562.07 mg/100g dw). In the body, phosphorus is closely linked to calcium, both contributing to the formation and solidification of bones. Food is "good" if the Ca/P ratio is at most 0.5. This increases calcium absorption in the small intestine (Olafoe *et al.*, 2009).

**Table 4** Total antioxidant capacity (mgEq.catechine /g dw)

Specie	Agroecological Zone	Regions	# Total antioxidant capacity (mgEq.catechine/g dw)
<i>L. siceraria</i>	High Plateau of the West	North West	92.15 ± 39.52
		West	83.61 ± 17.03
		<b>Average ± SD</b>	<b>87.88 ± 17.57<sup>bc</sup></b>
<i>C. mannii</i>	High Plateau of the West	North West	92.75 ± 16.38
		West	71.34 ± 17.89
		<b>Average ± SD</b>	<b>82.05 ± 9.90<sup>bc</sup></b>
<i>C. maxima</i>	High Plateau of the West	North West	68.40 ± 24.07
		West	65.17 ± 16.77
		<b>Average ± SD</b>	<b>66.79 ± 11.98<sup>ab</sup></b>
<i>C. moschata</i>	High Plateau of the West	North West	50.84 ± 12.69
		West	50.54 ± 22.66
		<b>Average ± SD</b>	<b>50.69 ± 10.60<sup>a</sup></b>
<i>C. sativus</i>	Humid Forest	Centre	99.53 ± 22.61
	Swampy Forest	South West	96.04 ± 37.51
	<b>Average ± SD</b>		<b>97.78 ± 17.88<sup>c</sup></b>

# = No significant difference between samples from different areas of cultivation

Fisher Test: Values with the same letter superscript within a column are not statistically different (p <0.05).

**Table 5** Antiradical activity of Cucurbit oils

Specie	Agroecological zone	Region	Effective concentration 50 (mg/ml)	# Antiradical activity (ml/mg)	
<i>L. siceraria</i>	High Plateau of the West	North West	11.47±0.80	0.088±0.006	
		West	12.92±1.25	0.078±0.008	
		<b>Average ± SD</b>	<b>12.19±0.61</b>	<b>0.083±0.004<sup>d</sup></b>	
<i>C. mannii</i>	High Plateau of the West	North West	24.37±2.82	0.041±0.005	
		West	19.66±4.28	0.053±0.012	
		<b>Average ± SD</b>	<b>22.01± 2.09</b>	<b>0.047±0.005<sup>b</sup></b>	
<i>C. maxima</i>	High Plateau of the West	North West	34.69±3.45	0.029±0.003	
		West	26.46±4.83	0.039±0.007	
		<b>Average ± SD</b>	<b>30.58±2.42</b>	<b>0.034±0.003<sup>a</sup></b>	
<i>C. moschata</i>	High Plateau of the West	North West	35.15±17.88	0.033±0.015	
		West	38.45±6.71	0.027±0.005	
		<b>Average ± SD</b>	<b>36.80± 7.80</b>	<b>0.030± 0.007<sup>a</sup></b>	
<i>C. sativus</i>	Humid Forest	Centre	18.89±1.56	0.053±0.005	
	Swampy Forest	South West	15.97±0.44	0.063±0.001	
	<b>Average ± SD</b>		<b>17.43±0.66</b>	<b>0.058±0.002<sup>c</sup></b>	
Olive oil			<b>Average ± SD</b>	<b>10.47±0.36</b>	<b>0.095±0.003<sup>d</sup></b>

# = No significant difference between samples from different areas of cultivation.

Fisher test: values with the same superscript letter within a column are not statistically different (p <0.05).

### The Mineral Content

Generally, the mineral contents of these Cucurbit seeds depend on the specie and not on the area of cultivation, except calcium and iron which varied according to the area of cultivation.

This ratio is less than 0.5 in these Cucurbit seeds, suggesting a good absorption of the calcium they contain. According to FAO/WHO (2001), the Recommended Nutrient Intake (RNI) is the daily intake, which meets the nutrient requirements of almost all (97.5%) apparently healthy individuals in an age and

sex specific population group. The RNI for calcium are 500-700mg (Children, 1-9years); 1000-1300mg (9years and above). The calcium levels of these seeds (48-90mg) are low, to be considered as calcium sources to cover the RNI for calcium. Phosphorus and calcium are essential for the development of bones and teeth preventing abnormal development of the skeleton. Their levels in these seeds can however, contribute to the health of individuals especially in children.

The **magnesium** levels range from 371.62 (*L. siceraria*) to 586.21 mg/100g dw (*C. moschata*). The value for *C. maxima* (462.96) is higher than that of *C. maxima* (364.43 mg/100g dw) reported by Alfawaz (2004). The RNI for magnesium are 53mg (infants, 7-11 months); 60-100mg (Children, 1-9years); 230-250mg (10-18 years); 190-260 (adults) and 220-270mg (pregnancy-lactation) (FAO / WHO, 2001). The magnesium levels of these seeds (371-586mg) are quite high, indicating that these seeds could be used to cover the RNI for magnesium. Magnesium is essential for the metabolism of calcium, proper functioning of the heart and is also involved in the metabolic processes of energy production as a cofactor for some enzymes. Given that high magnesium intake can increase parasitemia, for magnesium is equally a cofactor for the energy metabolism of *Plasmodium* (Ponka, 2006), therefore in malaria endemic zones, these seeds could be used as magnesium sources, after a malaria attack (after reduction of the *Plasmodium* load).

The **Potassium** levels range from 538.91 (*L. siceraria*) to 681.38 mg/100g dw (*C. moschata*) seeds. These values are higher than that of *C. maxima* seeds (98) but lower than that of watermelon seeds (1176 mg/100g dw, by El-Adawy and Taha (2001). The **sodium** content varies from 1.27 (*C. maxima*) to 2.01 mg/100gdw (*C. moschata*). The sodium content of *L. siceraria* (1.49) is less than that of *L. siceraria* (30 mg/100g dw, Olaofe et al., 2009) and watermelon seeds from Egypt (33 mg/100g dw, El-Taha and Adawy, 2001). Potassium, sodium and chlorine are the main electrolytes of biological fluids which maintain osmotic pressure (Latham, 2001). The Na/K ratio is of great importance in the prevention of hypertension. A ratio of < 1 is generally recommended (Olaofe et al., 2009). The Na/K ratio of these seeds is < 1. They therefore have potential for the prevention and dietary management of hypertension. The Recommended Daily Allowance (RDA) is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97-98%) of healthy individuals.

The United States Food and Nutrition Board established RDA for 3 macrominerals: Calcium (1000mg for adult men and women), magnesium (420 mg for adult men and 310-320 mg for women) and phosphorus (700 mg for adult men and women) (Crawford, 2011). The results obtained from these seeds (371-586mg) show that they can all be used to cover the RDA for magnesium while *C. moschata* can be used to cover the RDA for Phosphorus.

#### Trace Elements (Microminerals)

Table 7 shows that the trace elements, iron and zinc are predominant. The **iron** levels vary from 4 (*C. sativus*) to 5.06 (*C. moschata*) seeds which have the highest levels, similar to those of *C. mannii* (4.82) and *C. maxima* seeds (4.68 mg/100g dw). The iron content of these seeds is lower than that of watermelon seeds (12.1 mg/100gdw, El-Adawy and Taha, 2001). Iron is a key component of hemoglobin, which transports and distributes oxygen in the body. Iron deficiency is common in children and affects psychomotor development (Ekweagwu, 2008). Food is rich in iron when it contains 16mg of iron/100g of food (FAO/WHO 2001). These seeds therefore have low iron levels. However, they can be used to enhance the health of people living in malaria endemic zones, during and especially after a malaria attack. This is because, *Plasmodium* which is responsible for malaria, penetrates into erythrocytes (rich in iron) for their development, during a malaria attack. Less iron intake is therefore protective during *Plasmodium* attack, for the parasite feeds on erythrocytes. At the end of the crisis, the erythrocytes should be enriched with iron through consumption of iron containing foods like Cucurbit seeds (Ponka, 2006).

The **zinc** content varies from 2.66 (*C. sativus*) to 5.46 mg/100g dw (*C. moschata*). These values are higher than that found by Alfawaz (2004) in *C. maxima* seeds (1.09 mg/100 g dw). Zinc is an ubiquitous trace element. In metabolism, zinc plays an important role in the transcription of DNA, translation of RNA and cell division. It is very important for the proper functioning of the immune system. Zinc deficiency contributes to increased incidence and severity of infections such as malaria, diarrhoea and pneumonia (Ekweagwu, 2008).

**Table 6** Contents in Macrominerals (Calcium, Phosphorus, Magnesium, Potassium and Sodium) per region (mg/100g dw)

Specie	Agroecological zone	Region	*Ca	#P	#Mg	#K	#Na
<i>L. siceraria</i>	High Plateau of the West	North West	49.19±3.10	522.70±17.15	340.93±20.64	500.54±17.49	1.25±0.43
		West	63.71±11.04	625.12±83.30	402.30±83.14	577.28±88.64	1.73±0.46
		<b>Average ± SD</b>	<b>56.45±4.68<sup>a</sup></b>	<b>573.91±34.72<sup>ab</sup></b>	<b>371.62± 34.97<sup>a</sup></b>	<b>538.91± 36.89<sup>a</sup></b>	<b>1.49±0.26<sup>ab</sup></b>
<i>C. mannii</i>	High Plateau of the West	North West	85.87±19.56	663.59±168.34	431.67±123.19	654.60±169.63	1.90±0.23
		West	89.73±11.21	692.09±49.69	454.21±46.99	701.99±59.92	1.73±0.40
		<b>Average ± SD</b>	<b>87.80±9.20<sup>b</sup></b>	<b>677.84±71.65<sup>c</sup></b>	<b>442.94±53.83<sup>bc</sup></b>	<b>678.30±73.44<sup>b</sup></b>	<b>1.81±0.19<sup>bc</sup></b>
<i>C. maxima</i>	High Plateau of the West	North West	46.01±3.05	464.04±79.94	464.14±59.69	611.14±81.30	1.36±0.26
		West	50.65±1.91	641.06±35.64	461.78±19.70	563.79±48.75	1.19±0.12
		<b>Average ± SD</b>	<b>48.33±1.47<sup>a</sup></b>	<b>652.55±35.73<sup>bc</sup></b>	<b>462.96±25.66<sup>c</sup></b>	<b>587.47±38.70<sup>a</sup></b>	<b>1.27±0.12<sup>a</sup></b>
<i>C. moschata</i>	High Plateau of the West	North West	46.87±10.03	763.69±38.74	563.78±56.23	642.17±48.13	2.00±0.38
		West	55.45±11.86	856.83±58.22	608.64±64.94	720.58±54.21	2.03±0.26
		<b>Average ± SD</b>	<b>51.16±6.34<sup>a</sup></b>	<b>810.26±28.55<sup>c</sup></b>	<b>586.21±35.07<sup>d</sup></b>	<b>681.38±29.60<sup>b</sup></b>	<b>2.01±0.19<sup>c</sup></b>
<i>C. sativus</i>	Humid Forest	Centre	92.02±1.51	579.01±13.98	384.87±24.51	602.78±11.81	1.06±0.03
	Swampy Forest	South West	88.10±9.32	545.13±21.61	376.31±5.26	596.45±3.53	1.83±0.57
		<b>Average ± SD</b>	<b>90.06±3.85<sup>b</sup></b>	<b>562.07±10.51<sup>a</sup></b>	<b>380.59±10.24<sup>ab</sup></b>	<b>599.62±5.03<sup>ab</sup></b>	<b>1.45±0.23<sup>ab</sup></b>

\* = Significant difference between samples from different agro-ecological zones within a column (p < 0.05)

# = No significant difference between samples from different areas of cultivation within a column

Fisher test: values with the same letter superscript within a column are not statistically different (p < 0.05).

*L. siceraria* has the highest copper content (0.59) while *C. maxima* (0.11) and *C. moschata* seeds (0.15 mg/100g dw) have the least. The copper contents of these seeds are lower than those of *C. maxima* (1.7) and watermelon seeds (2.1mg/100g dw), reported by El-Adawy and Taha (2001). The copper level of *L. siceraria* could meet up the Recommended daily allowances of 1-8 year old children (0.34-0.44mg/day), but lower for the other age groups (RDA of 0.7-1.3mg/day) (FAO WHO, 2001). Copper is essential for the transport of iron in the body. Its deficiency is associated with susceptibility to infections and microcytic anemia. Alongside with manganese, copper is a cofactor of cytosolic superoxide dismutase, one of the enzymes involved in the fight against oxidative stress (Arredondo and Nunez, 2005).

After iron and zinc, manganese (Mn) is the 3rd most abundant trace element (among those analysed) in these seeds, with levels varying from 1.69 (*L. siceraria*) to 2.81 mg/100g dw (*C. moschata*). The adequate intake of Mn for individuals above one year is from 1.2 -2.6mg /day (FAO/WHO, 2001). These Cucurbit seeds could therefore provide an adequate amount of manganese required for the proper functioning of the immune system, growth of bones and with vitamin K, promote blood clotting (Aschner and Aschner, 2005).

The Food and Nutrition Board has established an RDA for the following trace minerals for adults: Iron (8mg for men and 18mg for women); zinc (0.011mg for men and 0.008mg for women) and copper (0.9mg for men and women) (Crawford, 2011). The results obtained from these seeds show that they are low in iron (3 - 5.06mg), high in Zinc (2 - 5.46mg) and low in copper (0.11 - 0.59mg). Considering the importance of zinc in enhancing the functioning of the immune system, these seeds could be used as sources of zinc, especially for people living with HIV/AIDS, to cover the RDA for zinc. The body depends on a regular zinc supply provided by the daily diet because stores are quite limited. Food diversity analysis demonstrates that it is virtually impossible to achieve zinc adequacy in the absence of a flesh food source (FAO/WHO, 2001). The consumption of Zinc-rich foods would perhaps decrease the prevalence of stunting in many developing countries with low-zinc diets, because linear growth is affected by zinc supply.

On the whole, the mineral contents highly depend on the specie. These seeds can be classified according to their mineral composition as follows: *C. moschata* > *C. mannii* > *C. maxima* > *L. siceraria* > *C. sativus*.

## CONCLUSION AND RECOMMENDATIONS

This study which was aimed at identifying secondary metabolites, quantifying total phenols, evaluating the total antioxidant capacity and the antiradical ability, and at determining the mineral content of five Cucurbitaceae seeds shows that:

Saponins, tannins, flavonoïds, alkaloids, phenols and triterpenes were present in all the seeds, except *C. mannii* which did not contain saponins. *C. sativus* had the highest level of tannins. All the seeds showed total antioxidant capacities, with *C. sativus* having the highest. The antiradical activity of the oils extracted from these seeds showed that all the oils trap the DPPH• radical, but with varying efficiencies, with *L. siceraria* seed oil having the highest antiradical activity, which was not significantly different from that of olive oil. Considering their antioxidant capacity (ability to reduce the formation of free radicals, especially *C. sativus*), high antiradical activity (ability to trap free radicals, especially *L. siceraria*), low atherogenic ratio (ability to lower LDL cholesterol and increase HDL-cholesterol levels, especially *C. sativus*) these oils are to be considered in the prevention and fight against cardiovascular diseases, urinary disorders (cystitis) and prostate cancer (benign prostatic hyperplasia).

The macrominerals and trace elements analyzed were found in all the seeds. *C. moschata* had the highest levels of phosphorus, magnesium, potassium, sodium (macrominerals). *C. sativus* had the highest level of calcium followed by *C. mannii*. The calcium levels were generally low and the Ca/P ratio of these seeds was less than 0.5, suggesting a good absorption of this calcium in the small intestines. The magnesium levels of these seeds were quite high, indicating that these seeds could be used to cover the Recommended Nutrient Intake for magnesium in all individuals from 7 months and above. These seeds generally had average potassium and low sodium levels.

**Table 7** Contents in Trace elements (Iron, Zinc, Copper and Manganese) per region (mg/100gdw)

Specie	Agroecological zone	Regions	*Fe	#Zn	#Cu	#Mn
<i>L. siceraria</i>	High Plateaus of the	North West	3.58±0.14	3.34±0.11	0.54±0.22	1.64±0.20
	West	West	4.42±0.34	4.27±0.57	0.64±0.17	1.74±0.16
	<b>Average ± SD</b>		<b>4.00±0.24<sup>a</sup></b>	<b>3.80±0.24<sup>b</sup></b>	<b>0.59±0.11<sup>c</sup></b>	<b>1.69±0.11<sup>a</sup></b>
<i>C. mannii</i>	High Plateaus of the	North West	4.58±0.39	3.19±0.69	0.30±0.06	2.58±0.41
	West	West	5.06±0.29	3.36±0.21	0.34±0.02	2.69±0.10
	<b>Average ± SD</b>		<b>4.82±0.20<sup>b</sup></b>	<b>3.28±0.29<sup>ab</sup></b>	<b>0.32±0.02<sup>b</sup></b>	<b>2.64±0.17<sup>b</sup></b>
<i>C. maxima</i>	High Plateaus of the	North West	4.78±0.68	4.26±0.66	0.11±0.01	2.59±0.59
	West	West	4.59±0.21	3.42±0.34	0.12±0.01	2.36±0.06
	<b>Average ± SD</b>		<b>4.68±0.29<sup>b</sup></b>	<b>3.84±0.30<sup>b</sup></b>	<b>0.11±0.005<sup>a</sup></b>	<b>2.47±0.25<sup>b</sup></b>
<i>C. moschata</i>	High Plateaus of the	North West	4.78±0.32	4.91±1.04	0.17±0.08	2.81±0.31
	West	West	5.34±0.51	6.01±1.34	0.13±0.01	2.80±0.56
	<b>Average ± SD</b>		<b>5.06±0.25<sup>b</sup></b>	<b>5.46±0.69<sup>c</sup></b>	<b>0.15±0.03<sup>a</sup></b>	<b>2.81±0.26<sup>b</sup></b>
<i>C. sativus</i>	Humid Forest	Centre	3.95±0.29	2.73±0.09	0.37±0.04	2.08±0.05
	Swampy Forest	South West	3.41±0.40	2.59±0.15	0.39±0.01	2.00±0.02
	<b>Average ± SD</b>		<b>3.68±0.20<sup>a</sup></b>	<b>2.66±0.07<sup>a</sup></b>	<b>0.38±0.02<sup>b</sup></b>	<b>2.04±0.02<sup>a</sup></b>

\* = Significant difference between samples from different agro-ecological zones within a column (p <0.05)

# = No significant difference between samples from different agro-ecological zones within a column

**Fisher test:** values with the same letter superscript within a column are not statistically different (p <0.05).

The potassium levels were slightly lower than the Recommended Dietary Allowance for adult men and women (700mg/day) but the Na / K ratio is < 1, which is generally recommended for the prevention and dietary management of hypertension. For the microminerals, iron, zinc and manganese predominate, followed by copper. *C. moschata* had the highest level of these trace elements analysed except copper with highest levels in *L. siceraria*. However, these seeds generally had low iron (< 16mg/day for foods rich in iron), and copper levels but high in Zinc (values can cover the RDA of 0.011mg for men and 0.008mg for women) and Manganese levels (can cover the RDA of individuals above one year). The ground paste of these seeds (especially *C. moschata*) can be added to diets as magnesium, zinc and manganese supplements to reduce their deficiencies especially for people living with HIV/AIDS (in order to enhance the immune system).

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