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Seeds Oils of the Most Consumed Cucurbitaceae in Benin: Phytochemistry and Antimicrobial Activities

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ABSTRACT

Citrullus lanatus, *Cucumeropsis edulis* and *Lagenaria siceraria* are three species of squash widely cultivated and used in food in Benin. The aim of this study is to determinate the physicochemical characteristic and antimicrobial activities of their seeds oil. Oils were obtained by Soxhlet extraction; their quality index were evaluated according to the methods listed in the ''AFNOR''. The oil yield of the seeds of the three cucurbits ranged from 36.23 to 42.28%, the moisture contents of the seeds oils ranged from 0.47 and 0.98%. In terms of quality index, the values are between: 5.04-14.58 mg/g for acid value, 31.70 - 31.86 % for iodine, 1.65 - 8.42 meqO_2/Kg-oil for peroxide value, 50.49 - 75.73 mg KOH/g-oil saponification and for ester 40.39 - 70.68%. Their calculated calorific value varies between 44611.05 and 45717.65 Kj /Kg-oil). AAS revealed that these oils had low content of Iron, Manganese and Potassium and do not contain Sodium at all. HPLC showed that the three oils are good sources of fat-soluble vitamins (A, E and K1) with 6.18 mg/100g vitamin E in *Citrullus lanatus* seeds oil and K1 (0.83 mg / 100g). They were all bacteriostatic and fungistatic on the strains explored but *Citrullus lanatus* and *Cucumeropsis edulis* oils had an antibiotic power on *Escherichia coli* and *Staphylococcus aureus*.

Introduction

In Africa, the use of vegetable seed for food dates back to antiquity. Cucurbitaceae is one of the sources of those seed. Species of this family are present on all continents but they are widespread in the subtropical and tropical regions. They are commonly called "pistaches" in Cote d'Ivoire or "Egusi" in Benin and Nigeria [1]. In Benin, three species are widely grown by farmers for their high protein and fat content *Citrullus lanatus*, *Cucumeropsis edulis* and *Lagenaria siceraria* [2, 3]. Almonds extracted from the shelled seeds of these species are reduced to paste to thicken the sauce or to make meatballs in the sauce [4]. Their lipids content varies but remain comparable to that peanut for example *C. lanatus* contained 40 - 44 % of lipids, *C. edulis* 44 - 45 % et *L. siceraria* 40 - 46 % [5,6,7]. So, the use of dough or flour of these seeds in sauces no longer requires the addition of oil during cooking. Several studies have been carried out on fatty acid composition of these three Cucurbitaceae [8, 9, 10, 11], but few was focused on their biological property. The knowledge of specific properties of oils contained in Cucurbitaceae seeds is necessary for their better valorization and use. It is also in the context that this paper examines the physicochemical characteristics and the antimicrobial power of seeds oils of the three Cucurbitaceae species most used in Benin.

Materials and methods

Collection of plant material

The main material consists of three Cucurbitaceae seeds: *Citrullus lanatus*, *Cucumeropsis edulis* and *Lagenaria siceraria*.

The three species seeds were obtained from Houndjro market in Abomey town, Zou department, Benin Republic and were identified at Abomey-Calavi University National Herbarium. The dried seeds were manually dehulled and drying at room temperature in laboratory.

Oil extraction

Oil was extracted with hexane using Soxhlet-type extractor for 6 hours at 69 ° C. Solvent was recycled to the rotary evaporator and the crude vegetable oils were dried in an oven (20 min, 103 ° C), cooled with the desiccator (30 min) and weighed. Extracted oils were packaged in dark bottles prior to analysis.

Physicochemical characterizations of oils

Physicochemical characteristics of the three oils were determined as in: [12] for Acid number, [13] for Saponification index, **[14]** for Peroxide index, **[**15**]** for Iodine index and Ester index according to [16].

Determination of mineral content

Iron, Potassium, Sodium, Magnesium were determined by Atomic Absorption Spectrophotometry (AAS) and flame photometer **[**17**].**

Determination of fat soluble vitamins

Vitamins A, E and K1 quantification was carried out by standard superposition method. An Agilent Technologies HPLC system (1120 Compact LC) equipped with an isocratic pump Isopump G4286A), a loop injector (Rheodyne P/N 5067- 4102) and a UV-Visible detector (G4286A) was used. Analytical conditions were: a column C18 (Hypersil ODS (5 µm), 150 x 3.9 mm), mobile phase Methanol, grade HPLC,

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flow rate : 0.5 ml /mn, pressure 36.9 bars, running time Detection wave length: 280 nm .Total run time required was equal to 10 min.

Evaluation of antimicrobial activity

The microbial support used is made up of: *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923 and *Candida albicans* 10231, *Salmonella tiphy*. These strains were provided by the National Laboratory of Public Health of Health Ministry of Benin. The method used is the one reported by **[**18, 19**]** using the microplates with 96 wells and Muller Hinton Broth (MHB) added with phenol red 0.02 g/L. A negative control made of a mixing of tested oil and the medium MHB and a positive control carried out with a mixing of tested microorganism and the MHB without oil. The cultured microplates are incubated at 37 ± 1 °C for 24 hours.

Results and Discussion

Table 1. Physicochemical properties of the three squashes seeds oils.

Parameters	***C. lanatus***	***C. edulis***	***L. scieraria***
Lipid content (%)	42.28 ±1.28	40.56 ±0.39	36.23±0.83
Moisture content (%)	0.62 ±0.02	0.47 ± 0.00	0.98 ± 0.09
Acid value (mg KOH/g)	10.09±1.12	14.58±1.12	5.04 ± 0.56
Iodine value (%)	31.74 ± 0.00	31.86 ± 0.01	31.70 ± 0.00
Peroxyde value (meq/Kg oil)	1.65 ±0.04	6.25±1.25	8.42 ± 1.25
Saponification value (mg KOH/g oil)	50.49±5.61	72.93±5.61	75.73 ± 2.80
Color	Yelow	Yelow	Yelow

Physicochemical properties of the three seeds oils are presented in Table 1. The oil yields of the three seeds were round 36.23 and 42.28 % by solvent extraction, these values were close to those obtained with Nigeria *Cucumeropsis edulis* (38.85 - 40.26- %) *Lagenaria siceraria* (40.86%) and *Citrullus lanatus* (57.26%) **[**20, 21, 22**]**. And were higher than the value reported by **[**23] on Botswana and Ghana *Citrullus lanatus* seeds oils (24.8%), this could be to the cultivars used. The three oils had the lowest values (0.47 - 0.98%) for moisture contents which indicates that these oils can be stored for a long period of time. Acid value of oils ranged from 5.04 - 14.58 mg KOH/g oïl. The acid values of the three oils were higher than Codex Alimentarius values for virgin vegetable oils. Acid value represents free fatty acid content due to enzymatic activity. Oils usually contain small amounts of free fatty acids such that when exposed to the air, these fatty acids, which are responsible for the acidity and oxidability of oils, produce unpleasant odors. In refined vegetable oils, the lower the free fatty acid content the more stable the oil, the more acceptable the oil to the human palate **[**24**]**. This will not be the bus for the three oils studied here. Saponification values ranged from 50.49 (*Citrullus lanatus*) to 75.73 mg KOH/g oïl (*Lagenaria siceraria*); they are less than that of palm oil (190 – 209 mg KOH/g oil), peanut oil (187 – 196 mg KOH/g oil) and cotton oil (189 - 198 mg KOH/g oil) **[**24**]**. The low saponification values as in the case of the three oil, indicate the predominance of long chain fatty acids [25, 26]. Peroxide values (1.65 - 8.42 meq/Kg oil) of the three oils are all below the limit value of the standard Codex Alimentarius (10 meq/Kg oil). Peroxide value depends on a number of factors such as the state of oxidation and the type of fatty acids present in the oil. Also, the high peroxide values may be due to causing lipid oxidation resulting from the absorption of oxygen, which increases the formation of peroxides [27**]**. The low peroxide content of *C. lanatus* seed oil is indicative of the fact that this oil is not likely to deteriorate compared to the other two oils. Iodine value of the three oils is around 31.74 less than that of palm oil (50 - 55), peanut oil (77 – 107) and cotton oil (100 - 123) [24]. Iodine value of oil indicates its unsaturated fatty acids content and explains its liquid state; this justifies the fluidity of the three squashes seed oils compared to the palm, peanut and cotton oils widely used in Benin.

For minerals content (Table 2), none of the three oils contain sodium, potassium was only found in *C. lanatus* seed oil, iron in *C. lanatus* and *C. edulis* seed oil and magnesium was present in the three oils. Potassium is the principal cation in intracellular fluid while sodium is the principal cation in extracellular fluids, magnesium is found in almost all enzymes where thymine pyrophosphate serves as a cofactor, it is also found as a component of kinase enzymes, teeth and bones [17]. Iron is important for human health but iron content of oil is strongly correlated with its stability because it is a pro-oxidative metal like copper [28, 29] and would lead to a degradation of the nutritional value of the products.

Table 2. Mineral content of the three squashes seeds oils.

Mineral content (ppm)	***C. lanatus***	***C. edulis***	***L. scieraria***
Iron (Fe)	0.32	0.21	nd
Magnesium (Mg)	1.14	0.11	0.03
Sodium (Na)	nd	nd	nd
Potassium (K)	0.06	nd	nd

With regard to the vitamin content, the the three oil samples contained vitamins A, E and K1 (Table 3). *C. lanatus* seed oil had the lowest vitamin A content but a high vitamin E and K content compared to the other two oils. Vitamin E is a collective term for tocopherols and tocotrienols, which are natural antioxidants that prevent the rancidity of oils during storage and thus delay its shelf-life. Some authors reported that vitamin A has anti retinitis properties [30]. Vitamin E plays an important role as one of the antioxidants **[**31**]** and has anti-inflammatory properties [32]. Vitamin K has long been known for its role in coagulation but has been recognized for its important role in maintaining bone strength and prevention of soft tissue calcification **[**33**]**. Vitamin K1 (Phylloquinone) from green leafy vegetables and vegetable oil represents the major dietary source of vitamin K for humans **[**34**]**. The three oils particularly *C. lanatus* seed oil can be used as dietary supplements to treat vitamin E and K deficiency and consequently fight against certain associated pathologies.

With regard to the antimicrobial properties, Minimum Inhibitory Concentration (MIC) of the three oils ranged from 6.25 mg / mL to 25 mg / mL for *Escherichia coli* ; from 12.5 mg / mL to 25 mg / mL for *Staphylococcus aureus* and *Candida albicans* ; 6.25 mg / mL to 12.5 mg / mL for *Salmonella tiphy*. All of them were bacteriostatic and fungistatic on the strains tested. Their Minimum Bactericidal Concentration (MBC) range from 25 to 50 mg / mL for *E.*

Table 3. Vitamins content of the three squashes seeds oils.

		Vitamins content (mg/100g)		
Retention Time (mn)	**Vitamins**	***C.lanatus***	***C. edulis***	***L.siceraria***
2.510	Vitamin A	1.01±0.02	1.48±0.01	1.49±0.00
5.257	Vitamin E	6.18±0.16	0.36±0.02	0.31±0.01
8.303	VitaminK_1	0.83±0.08	0.40±0.00	0.40±0.01

Table 4. Antimicrobial activities of the three species squashes seeds oils.

Seed oils	Strains tested											
	E. coli			*S. aureus*			*S. tiphy*			*C. albicans*		
	MIC	MBC	ap	MIC	MBC	ap	MIC	MBC	ap	MIC	MBC	ap
C. lanatus	6.25	25	4	12.5	25	2	6.25	>50	ND	12.5	>50	ND
C. edulis	12.5	50	4	12.5	12.5	1	12.5	>50	ND	12.5	>50	ND
L. siceraria	25	25	1	25	25	1	12.5	>50	ND	25	>50	ND

MIC: Minimum Inhibitory Concentration (mg/mL);CMB: Minimdal Concentration (mg/mL). Ap : antibiotic power ; ND = not detected

coli and from 12.5 mg / mL to 25 mg / mL for *S. aureus*, while CMB are greater than 50 mg / mL for *S. tiphy* and *C. albicans* (Table 4). In addition, they had an antibiotic power (ap) on *Escherichia coli* ($1 \leq ap \leq 4$) and on *Staphylococcus aureus* ($1 \leq$ ap ≤ 2), two bacteria involved in multiple infections in food contaminations [35, 36]. It is interesting to note that seed oil from this plant also sensitizes *S. Aureus* a strain commonly associated with the development of methicillin resistance. The use of these oils is therefore an asset that consumers can use as additives to foods and food formulations to ensure their microbiological quality.

Conclusion

Results of the study reveal that the three most consumed Cucurbitaceae seed had good lipid content. The oils are low in minerals and not soded, however they are sources of fat-soluble vitamins with sample A, the richest in vitamins E and K1. Only *Citrullus lanatus* and *Cucumeropsis edulis* seed oil behavedas antibiotics on *Escherichia coli* and *Staphylococcus aureus*. These oils can be consumed in the form of food supplements to fight against microbial contamination of foodstuffs and vitamin A deficiency

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