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Research Article

Phytochemical Composition and Antioxidant Power of *Citrullus colcynthis* from Togo

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ABSTRACT
Underdeveloped and developing countries suffer from Nutrition difficulties that require immediate attention. Consumption of food affects physical and mental health of a person. This study evaluates the chemical constitution and antioxidant capacity of *Citrullus Colocynthis*, the main source of daily food of the Togolese. The spectrophotometric evaluation of phenolic compounds (total phenolic, flavonoid, procyanidine and carotenoid) show that Citrullus *Colocynthis* has an important phenolic content (569, 5±0,010 mg GAE/g extract). The amounts of minerals (Calcium, Iron, Potassium, Magnesium, Lithium, Sodium and Copper) were also established by atomic absorption spectroscopy. The percentage of ash, protein and fat was also studied. Furthermore, Fatty acid and Amino acid in *Citrullus Colcynthis* were evaluated by chromatographic method. The total antioxidant activity was first estimated using the phosphomolybdenum method. In addition, four other methods were used to analyze the antioxidant activity of the methanol extract, as well as FRAP assay (ferric reducing antioxidant potential), DPPH radical scavenging assay, ABTS and *β*-carotene bleaching assay.

Keywords: Antioxidant Activity, GC-FID; HPLC-FLD, Mineral; phenolic contents Togolese Food

INTRODUCTION

The growing attention in the substitution of synthetic food antioxidants by natural ones has fostered research on vegetable sources and the screening of raw materials for identifying new antioxidants. Oxidation reactions are not an exclusive concern for the food industry, and antioxidants are widely needed to prevent atrophy of other oxidisable goods, such as cosmetics, pharmaceuticals and plastics. Systematic literature reviews on this subject indicate that TPC is considered as one of the major groups of composites acting as primary antioxidants or free radical terminators. It is therefore worthwhile to determine their total amount in the specific plant chosen for a study in addition; other biological properties such as anticarcinogenicity, antimutagenicity, antiallergenicity and antiaging activity have been reported for natural and synthetic antioxidants. Special interest is focused on their extraction from inexpensive or residual sources from agricultural industries. The interaction among free radicals, antioxidants, and co-factors is essential in maintaining health, aging and age-related diseases. Free radicals engender oxidative stress, which is liberated by the body's endogenous antioxidant systems with aninput from co-factors, and by the ingestion of exogenous antioxidants. Its leaves are taken before the onset of the first buds and are crushed and mixed with the flour of corn to prepare a dish similar to couscous. In traditional societies of the Togo savanna region, a roasted seed of *Citrullus Colocynthis* is an ingredient used during weddings, christenings and other popular ceremonies instead of groundnut. Furthermore, Citrullus Colocynthis is one of the most studied species in our countries due to its alleged pharmacological properties. It is, for example, recommended as an ingredient to make sauces after undergoing surgery or a blood test or to be fed to women after childbirth and during painful menstruation. In addition, this paste is included in children's diets when their eyes have turned whitish, a Recognizable sign of anemia for the people living in this area. Therefore, in this paper we will present the phytochemical constituent and their antioxidant capacity.

MATERIALS AND METHODS

Plant material

Particularly in Dapaong, Latitude 10°53'N, Longitude 00°15'E,Longitude 230 there is a Citrullus Colocynthis culture area located in the northern part of Togo. The leaves, alternating from 10 cm to 15 cm, have a long blade deeply sliced into 3-5 lobes. It is a monoecious plant, where the first flower buds, which are unisexual, solitary and axillary, generally appear in summer on the 34th or 35th day after sowing. Nevertheless, any vegetation dries out three months after the beginning of fructification, which takes about 55 days. The corolla of petals welded to the base has a diameter ranging from 3 to 3.5 cm. The fruits are oblong or pear-shaped berries 12 to 22 cm in diameter and vary from a variegated light green color to dark green when still green to yellow when ripe. Its seeds can be

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Table 1: phenolic content in Citrullus Colocynthis

	TPC (mgGAE/g extract)	TFC	Carotenoid	Procyanhidine	T-AOC
Content ±SEM	569,5±0,010	231,111±0,026	30,868±0,108	85,395±3,119	60,69962 ±0,096

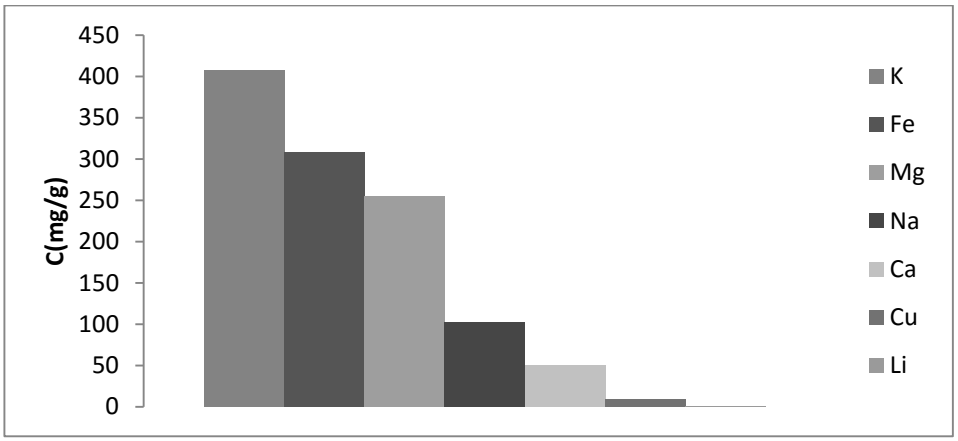

Figure 1: Minéral content in *Citrullus Colocynthis*

Table 2: Antioxidant capacity expressed as micromoles of trolox equivalents per gram

	DPPH	ABTS	FRAP
Eq trolox (µm/ml)	10.99±0,23	22.28±0,531	1,418±0,055

roasted and crushed, then mixed with corn flour (Zea mays) or pearl millet (pennisetum typhoid); the bark can be dried and grinded with Fogara zantholoides (Rutaceae) powder to prepare, when mixed with other spices like ginger and pepper, cakes which are marketed in some localities of the region of the Togo savanna.

Determination of the crude protein

This analysis was carried out using the distillation Micro Kjeldahl methods described by Oyeleke (1984)[1]. This process necessitates digestion, distillation and titration. The dust of Citrullus Colocynthis (25-30 mg) was mixed with 2g of a mixed catalyst (60g of CuSO4, 100 g of K_2SO_4 and 2 g of selenium dioxide) and then added to the samples. We add 6 mL of free concentrated sulphuric acid The mineralization of this combination was carried on for 2 hours at 240°C. In a 100 mL beaker containing 10 mL of 2% boric acid plus 2 drops of mixed indicators (a mixture of 6ml methyl red and12 mL of Bromo-cresol Bleu), 40mL of NaOH (40g/l) and 15mL of distilled water. After the distillation of each exemplification, the apparatus was washed assiduously with distilled water before the next sample was introduced in it.The distillate collected was titrated against 0.01 N HCl solutions until a faint pink color was obtained. The procedure was repeated for each sample. The percentage of nitrogen content of each sample was calculated according

Protein (%) = Nitrogen (%) × Conversion factor (6.25)

Determination of the mineral elements

mineral elements and the trace elements has been determinate from the dried plant samples were digested in concentrated HNO_3.The proximate assay was carried out using standard methods[2]. while the mineral constitution of each sample was resoluted by wet washing each sample, followed by flaming in a Perkin- Elmer atomic absorption spectrophotometer.

Fatty acid determination

Hexane/Isopropanol solvent can extract neutral lipids (triglycerides), called polar lipids (partial glycerides, free fatty acids, unsaponifiables and phospholipids). The extraction was conducted according to AFNOR NF V03-030/1991:50 g of garlic added to hexane/isopropanol solvent and let stand at least 2 hours, then filtered through filtered. After concentration, the solution was used for the isolation of methyl esters of fatty acids according to ISO 5509-1978 by adding about 40 ml of methanol, 0.5 ml of the methanol potassium hydroxide solution and boiling with reflux condenser. The esters obtained are extracted with heptane, concentrated and then analyzed by GC-FID.

Amino acid determination

Citrullus Colcynthis (500 mg) was ground in 4 ml of 6 M HCl. The mixture stored in oven at 105°C/24 h. Hydrolyze was stopped by adding 6ml NaOH (6N). The sample was Filtered and recuperate in vial which is stored at 4 ° C. Transformation of free amino acids was carried out using 1ml 0, 4 N borate buffer, pH 10 and 10µl of mercaptoethanol and the mixture diluted 1/10 and stored in a vial. The mixture was transferred into a 2.5 µl glass insert in an amber vial for chromatography. Amino acids were separated and quantified according to[3], Mobile phase A consisted of Acetonitrile –Methanol –water (45/45/10) Mobile phase B was Na_2HPO_4 2,75g/l pH = 6,5. The gradient was 10% A and 90% B (0 min), 100% A (35 min). Excitation at 340 nm and at 440nm the emission. Peaks were identified by matching retention time with those of standards and individual amino acids. All the standard amino acids were obtained from Sigma–Aldrich.

Antioxidant Capacity

The total antioxidant capacity (T-AOC) was evaluated with the phosphomolybdenum test as is cited in[4]. The antioxidant activity of the sample was determined using a standard curve of ascorbic acid. The reducing capacity of the extract was expressed as mg of ascorbic acid equivalents (AAE)/g of extract. Also, The ABTS assay

Table 3: Antioxidant activity of extract: IC50 of DPPH ABTS and β-carotene

	β-carotene	DPPH	ABTS
IC50 (mg/ml)	0,68±0,013	4.15 ± 0.91	9.92±0.54
IC50 standard	1,557±0,03 (mg/ml)	EC50Trolox= 45.642 µM	EC50Trolox= 221.02 µM

Table 4 : Fatty acid amount in Citrullus Colcynthis

Saturated fatty acid			Unsaturated fatty acid		
Caproic acid	C6:0	-	Palmitoleic acid	C16:1	-
Capric acid	C8:0	-	Oleic acid	C18;1	10,64032
Lauric acid	C12:0	-	Linoleic acid	C18:2 52	66.66226
Myristic acid	C14:0	-	Linolenic acid(GLA)	C18:3 7.6	-
Palmitic acid	C16:0	10,88530	Arachidonic acid	C20:4	-
Heptadecanoic acid	C17:0	-			
Stearic acid	C18:0	11,81212			
Heneicosanoic acid	C21:0	-			
Tricosanoic acid	C23:0	-			

was carried using the method described by[5]. The antioxidant activities were measured at 734 nm in a Perkinelmer spectrophotometer. With minor modifications in the concentration of ABTS and potassium persulphate solution as follow 7 mM and 2.45 mM The sample was mixed with chloroform /methanol (v/v). The result was expressed such as trolox equivalent antioxidant capacity (TEAC). The antioxidant activity of the *Citrullus Colocynthis* methanol extract was evaluated using the β-carotene linoleate model system. In addition, DPPH free radical commensurate evaluate the antioxidant activity of the extracts was measured with scavenging activity of the stable with the method described by[7] with slight modifications. Finally, FRAP is a simple direct test for measuring the antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used for plant extracts. According to[7]. All the assays were carried out in triplicate, the results expressed as mean values ± standard deviations and the extract concentration providing 50% antioxidant activity (EC50) was calculated from the graph of antioxidant activity percentage against extract concentration. And compared to standard

RESULTS AND DISCUSSIONS

Mineral content

Mineral concentrations in many plant foods are low relative to human requirements. In order to satisfy human needs of mineral consumption is to determinate the amount of minerals in food. Figure 1resume the bioavailability of the mineral and trace elements from *Citrullus*, the important amount were associated to potassium iron and Magnesium. The potassium amount were chiefly higher than Na, which is consider as a blessing from the nutritional point of view[8]. therefore, Citrullus Colcynthis attest us a great source of useful minerals for human nutrition.

Total phenolic content (TPC)

Systematic literature reviews on this subject indicate that TPC acting as primary antioxidants is deliberated as one of the major groups of composites or free radical terminators. The different contents of the phenolic class, which are flavonoids, carotenoids and procyanhidine, are summarized in Table 1. Flavonoids were the dominating compound of phenolic class in *Citrullus Colocynthis*.

The phenolic compounds may directly contribute to the antioxidative action[9]. These compounds found in Citrullus Colcynthis with an amount of up to 1.0 g is ingested daily as part of a fruit and vegetable rich diet[10]. TPC obtained from *Citrullus Colcynthis* possess a broad spectrum of chemical and biological properties including radical scavenging properties and they have been reported to have multiple biological effects, including antioxidant activity which is the purpose of the second part of this study. Also, it has been recommended that phenolic compounds, present in Citrullus Colcynthis has inhibitory effects on mutagenesis and carcinogenesis in humans, the hydroxyl groups of phenolic compounds assured the scavenging ability[9]. Besides, the antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors which is related to their ability to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction)[11].

Total antioxidant capacity

The total antioxidant activity was evaluated using five models. The phytochemical composition varied according to the different solvent extracts of each model as seen in the qualitative phytochemical analysis shown in Table 1.

This method depended on the presence of antioxidant agents which reduce Mo (VI) to Mo (V) and assure the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm[12]. In the case of Citrullus Colcynthis, the methanol extract showed a significant value of total antioxidant capacity of 60.69962 ±0,096 mg (ascorbic acid equivalent)/ g dry extract.

Reproducibility of ABTS, DPPH, FRAP and β-carotene

All analyses of this plant extract had no genotype time interaction, indicating that all the techniques gave a comparable ranking of antioxidant activity among clones within each determination time. Therefore, the DPPH and FRAP assays with methanol extract might have been different among the determination times. The equipment of other three methods is available. Another advantage of the DPPH and FRAP is that the extracts reacted rapidly (30 min), in comparison with other tests like β-carotene (2 h)

Table 5: Amino acid profile of Citrullus Colcynthis

Amino acid	Concentration in mg/100g
Aspartic	4,508
Glutamates	10,005
Serine +glumine+ Histidine	2,188
threonine+ Glycine + Arginine	8,366
Valine + Methionine	1,179
Lysine	0,668
Phénylalanine	0,751
Isoleucine	0,930
Leucine	2,703

and ABTS reaction took much longer (16 h). The TEAC of Citrullus Colocynthis extracts was evaluated with the DPPH,
ABTS and FRAP tests (Table 2).
The free radical scavenging activity determined by DPPH was 0.99 µM trolox/g of extract. For the Frap assay, the trolox equivalent was 1,418 µM trolox and the value determined by ABTS was 22.28 µM trolox (Table 2). Thus, ABTS detected the highest antioxidant capacity in methanol extract of Citrulus Colcynthis.

Comparison of IC50

These assays specified that the other three techniques yielded comparable results between clones. Different concentrations of trolox were used as standard antioxidants.The
IC50 value for the antioxidant activity of the samples (Table 3) demonstrate the amount of sample concentration needed to produce 50% free radical scavenging activity calculated using trolox as standard.
Hence, methanol extracts of Citrullus Colocynthis seeds showed the highest DPPH free radical scavenging activity based in the IC50 value which is inversely proportional to the free radical scavenging activity. Here, results clearly show that. a possible explanation for these differences is the fact that pro-oxidative substances don't affect the ABTS, DPPH, and FRAP methods, unlike the β-carotene/linoleic acid method[13]. These different can be associated to the various methods show the importance of applying more than one analytical method, when the objective is to assess A_{ox} A in a food matrix or even in the pure compound. Here, the result clearly proves that the methanol extract had the highest antioxidant activity when using the β-carotene method. In addition the A_{ox} A is higher than the standard (BHT).

Physicochemical characterization of seeds

The seeds contained 18.376 ± 0.476 % of protein, 5.7 % of ash and 22.5% of fat on a dry weight basis. The protein proportion was lower than that of other cucurbit seeds[14] such as buffalo gourd that contained 28.5-31.7% of protein[15]. But the Togolese plant content an amount of protein higher than in the Citrullus Colocynthis collected from the desert areas according to[16]. the high fiber content of the seeds, more than half of the total seed weight can be attributed us a cause of the lower protein content of in *Citrullus Colocynthis*. After using the full potential of this oil seed in food preparations,it is recommended to be handled further to remove or decrease the crude fiber content, therefore increasing the protein content.

Fatty acid determination

Table 4 resume the fatty acid content of crude *Citrullus Colcynthis* seed oils seeds. Its oil had high amounts of total unsaturated fatty acids like linoleic and oleic acids. The first class of fatty acid was associated to linoleic acid content is the highest in Citrullus Colcynthis in compared with four curcubitacea plants: Cucurbita pepo L (47.0%)[17]; Pumpkin (Citrullus maxima) seed oil (45.5%)[18], pumpkin seeds (54.6%)[19] and pumpkin (Cucurbita maxima) (34.77%)[20]. Consequently, these oils could be used as a good source of essential fatty acid. Also, Citrullus Colcynthis oil is rich in oleic acids, which give it other applications as edible cooking oil, as salad oil, or for the manufacture of margarine[21]. In addition the oil with high content of oleic acid is wanted in field of nutrition and high stability cooking and frying oil. The rapidity of oxidize of oils, used for deep-fat frying was associated to high degree of unsaturation. For example, linoleic acid oxidizes 50 times faster than oleic acid[22]. Therefore; industry of edible oil gave attention on high oleic vegetable oils which characterized by adequate oxidative stability destined in demanding applications such as frying[23]. The saturated fatty acids presented in Citrullus seed oil were palmitic and stearic acids. These results were in agreement with[24]. For the paint industry these ratio should reduce the rate of drying and crosslinking and could be an advantage.

Amino acid determination

Free amino acids, which exist naturally in food, determine the taste, flavor, and quality of various[25]. The whole-seed amino acid compositions of Citrullus Colcynthis are presented in Table 5. The first limiting amino acid is lysine. Glutamate was the dominant amino acid in Citrullus Colcynthis which **is** non-essential amino acid. In addition, the phénylanine, isoleucine leucine lysine valine and methionine which are essential amino acid had an amount higher than in milk[26]. There is a rapidly growing body of literature that proves the immune benefits of supplementation with specific nutrients, particularly during critical stages of development or disease states, when animals may have a higher demand for essential and nonessential nutrients. Such states include weaning, infectious diseases or chronic inflammatory conditions[27].

CONCLUSION

The *Citrullus Colocynthis* shows the high phenolic content and antioxidant potential of, a popular food among the Togolese.
It also provides useful information for the development of safe food products and additives with appropriate antioxidant properties. Establishing the antioxidant status of food will promote research on the identification and quantification of the active constituents of such herbs, which may help protect consumers against free radical damage and oxidative stress-related diseases. As a result, folk medicinal practices, that make use of medicinal herbs, will be strengthened. This study will be able to provide useful information for identifying foods which are rich in protective antioxidants and for the development of safe

food products and additives with the appropriate antioxidant properties. In addition, they are good sources of protein, fat and essential amino acids which will Requirements, Technical Report of a joint enhance proper growth of children and meet the daily amino acid needs of adults.

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