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Nutraceutical Assessment of Four *Amaranthus* Species from Burkina Faso

Ouedraogo Ibrahim[1], [1]Hilou Adama, [1]Sombie Pierre Alexandre Eric Djifaby,
[1]Compaore Moussa, [2]Millogo Jeanne and [1]Nacoulma Odile Germaine
[1]Laboratoire de Biochimie et de Chimie Appliquées (LABIOCA),
[2]Laboratoire de Biologie et Ecologie Végétale, UFR-SVT, Université de Ouagadougou,
09 BP 848 Ouagadougou 09, Burkina Faso

Abstract: The use of the amaranths as vegetables is developed in Burkina Faso. Most known are *Amaranthus dubius* Mart. Ex. Thell, *Amaranthus graecizans* L., *Amaranthus hybridus* L. and *Amaranthus viridis* L. *A. hybridus* is most used and abundantly cultivated, however the others are more or less wild. Theaim of the present study was to investigate the nutraceutical potentialities of fourAmaranthus species from Burkina Faso. The aqueous decoction extract of the four species contains the highest ofpolyphenols, flavonoids,flavonols and proteins contents. The aqueous decoction extracts were used for the evaluation of the antioxidant activity and xanthine oxidase inhibitory potentiality. The A. dubius showed the most potent antioxidant activity with a CE_{50} of 1.26±0.46, mgm2 the β-carotene bleaching method.*A. hybridus* showed the most potent antioxidant activity (66.99±1.65 µmolAEAC/g) in the DPPH radical scavengingmethod. The aqueous extracts of the four speciesshowed weak inhibition of the xanthine oxidaseat the concentration of 200 µg/mL.The four Amaranthus species are rich in microelements (Na, K, Ca, Mg, P, Fe, Mn, Zn and Cu) and carotenoids compounds. The anti-nutritionals factors were also quantified. The oxalate amount in these species is fairly high. The highest minerals contents and the antioxidant activity of the amaranths could explain theirlarge use by all the socio-economic strata of the population.These plants would constitute a source of dietetic antioxidants.

Key words: Amaranthaceae, antioxidant activity, oxalate, phenolic and minerals content, xanthine oxidase

INTRODUCTION

The food supply of certain substances which finds in the plants, the animals and the minerals kinds are one of the major prerequisites to the maintain health.The natural substances resulting from the vegetable kingdom are traditionally sources of food, drugs for the man (Nacoulma, 1996).

Amaranthus plants (Amaranthaceae) are spread throughout the world, growing under a wide range of climatic conditions and they are known to infest or to produce useful feed and food products (Rastrelli *et al*., 1995). The leaves of amaranth constitute an inexpensive and rich source of protein, carotenoids, vitamin C and dietary fibre (Shukla *et al*., 2006), minerals like calcium, iron, zinc, magnesium (Kadoshnikov *et al*., 2008; Shukla *et al*., 2006), and phosphorus (Ozbucak *et al*., 2007).The use of these vegetables in traditional medicine was also reported (Nsimba *et al.*, 2008; Nacoulma, 1996).

The amaranths are cultivated like vegetables for their edible sheets with the manner of spinaches and sometimes like decorative plants for their spectacular ear flowering.Amaranthus dubius, Amaranthus graecizans, Amaranthus hybridus and *Amaranthus viridis* are mainly used like cooked vegetables, only or in association between them or with other plants like *Hibiscus* sabdariff*a* (Malvaceae).

Amaranthus dubius is recommended like food plant having medicinal properties for the young children, the nursing mothers, and for patients suffering from anaemia, kwashiorkor and insomnia (Nacoulma, 1996). In Tanzania, the whole plant is used as drug against the stomachache. Ethanolic extract of *A. dubius* showed a good inhibition of lipoxygenase (CE50 = 69.4 µg/mL) at the concentration of 100 µg/mL (Uma and Odhav, 2008).

Amaranthus *graecizans* is also used like fodder for the cattle.In Mauritania, one prepares fine wafers with seeds, whereas in the west of the United States one crushes them to make flour of it. In Uganda, the sheets are used to treat tonsillitis but in Senegal; the sheets are used as vermifuge (Maundu and Grubben, 2004).

In Burkina Faso, Amaranthus hybridus is used in the affections of liver, of the knee ache and the macerated aqueous is used as vermifuge (worm of Guinea). It is also used for its nematicidesvertues, healing, diuretic and laxative in the children (Nacoulma, 1996). The aqueous

Corresponding Author: Ouedraogo Ibrahim, Laboratoire de Biochimie et de Chimie Appliquées, Université de Ouagadougou, Burkina Faso, Tel.: 0022676564037

extracts of A. hybridus showed a anti-anemia activity on rabbits treated with the phenyl hydrazine hydrochoride with the amount of 30 mg/kg (Ogbe *et al*.,2010). According to studies undertaken by Uma and Odhav (2008) the extract ethanolic extract tested at 100 μg/mL on lypoxygenase inhibition showed a strong inhibiting capacity (CE50 = 77.2 μg/mL).

The sheets and the young plant of *Amaranthusviridis* are also used as fodder for the cattle and like green manure. The sheets are diuretic and purgative and they are used as cataplasm (fresh or in dried powder) to treat ignitions, furuncles, abscesses, gonorrhea, orchite and hemorrhoids.The sheets would have febrifuge properties (Nacoulma, 1996). Ashes of the plants Amaranthusviridis are rich in potassium and they are sometimes used to make soap (Burkill, 2000). An enzyme, the phosphoenolpyruvate carboxylase was isolated from the sheets of *A. viridis* (Iglesias *et al*., 1986).

In the aim to valorize medicinal and food plants usesin Burkina Faso, we propose to evaluate and compare the nutraceutic potentialities through characterization and quantification of their phenolic, mineral and bioactive compounds of *Amaranthus dubius* Mart. Ex Thell, *Amaranthus graecizans* L., *Amaranthus hybridus* L. and *Amaranthus viridis* L. used to treat tonsillitis but in Senegal; the sheets are used as vermifuge (Maundu and Grubben, 2004).

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MATERIALS AND METHODS

Plant sample collection: The leaves of the four plants were harvested in Ouagadougou, capital of Burkina Fasoin June 2009.The Plants were identified by Prof. Millogo, botanist at the University of Ouagadougou.

Extract preparation: A crude distilled water extract was prepared by heating powdered plant (25 g) in a flask with 250 mL distilled water for 30 min whilst stirring. Similarly, 25 g of powdered plant was extracted successively with methanol in a Soxhlet apparatus for 24 h. The extracts were filtered and evaporated to dryness in a rotary evaporator. All the extracts were kept at 4ºC and were dissolved in water or solvent before use.

Nutritional factors evaluation methods:

Protein content: The protein content was estimate by using the method of Lowry *et al*. (1951). 0.6 mL of plantextract (EM, DA) was mixed with 3ml of the Lowry reagent (1 mg/mL). Mixture was incubated during 10 mns. It is then added 0.3 mL of the Folin-Ciocalteureagent (1N). The mixture is also homogenized and incubated during 30 mns and the absorbance was measured at 660 nm. The protein content was determined using standard curves for Bovine Serum Albumin (50-500 μg/mL). The test is carried out in triplet for each extract. The protein concentration of the vegetable extract is expressedas mg equivalent BSA for 100 mg of extract.

Mineral analysis: The mineral elements comprising sodium, calcium, potassium, magnesium, iron, zinc and phosphor were determined according to the method of Shahidi *et al.* (1999) and Nahapetian and Bassir (1975) with some modifications. Two (2.0) g of each of the processed samples was weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550ºC in a muffle furnace. The resultant ash was dissolved in 5.0 mL of HNO_3/HCl/H_2O (1/2/3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5.0 mL of de-ionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100.0 mL volumetric flask by filtration through WhatmanNo.42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS300, Perkin Elmer). A concentration of each element in the sample was calculated on percentage (%) of dry matter (mg/100 g sample). Phosphorcontent of the digest was determined colorimetrically according to the method described by Nahapetian and Bassir (1975).

β-Carotene and lycopene determination: β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried extract (100 mg) was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

Lycopene (mg/ 100 mL) = -0.0458 A663 + 0.372 A505 - 0.0806 A453

β-carotene (mg/100 mL) = 0.216 A663 - 0.304 A505 + 0.452 A453

The assays were carried out in triplicate; the results were mean values±standard deviations and expressed as mg of β-carotene/g and mg of lycopene/g of extract.

Functional activity assessment method:

Determination of the antioxidant activity with the b-carotene bleaching method: The antioxidant activity of the extracts was evaluated by the method of Mi-Yae *et al*. (2003) with some modification.A solution of β - carotene was prepared by dissolving 8 mg of β - carotene in 10 mL of chloroform.0.5 mL of this solution was introduced into a100 ml round-bottom flask.Chloroform is removed under vacumat 40ºC. Fourty seven (47) µL of linoleic acid, 362 µL of Tween 40 and 100 mL of distilled water were added. The mixture was shaken vigorously to form the emulsion. Aliquots (4.8 mL) of this emulsion are transferred in test tubes containing 0.2 mL of different concen trations of the extracts.

The tubes are shaken and incubated at 50ºC during 2 h The absorption at time zero is measured just after the addition of the emulsion then that at the end of the 2 h of incubation to 470 nm. The antioxidant activity was calculated by using the following equation:

$$AA = [1- (A_0 - A_t) / (A'_0 – A'_t)] \times 100$$

where, A_0 and A'_0 are the absorbance measured at zero time of incubation for the test sample and control, respectively, and A_t and A'_t are the absorbancemeasured in the test sample and control, respectively, after incubation for 2 h.

DPPH radical method: Ability of the extracts to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was evaluated as described by Lamien-Meda *et al*. (2008). Extracts were dissolved in methanol and 0.75 mL of each was mixed with 1.5 mL of a 0.02 mg/mL; solution of DPPH in methanol. The mixtures were left for 15 min at room temperature and the absorbance was measured at 517 nm. The blank sample consisted of 0.75 mL of extract solution with 1.5 mL of methanol. The antioxidant content was obtained, expressed as µmol of ascorbic acid equivalent per g of extract antioxidant content (µmol AEAC /g extract).

Xanthine oxidase inhibition: The inhibiting activity of the extracts on XO (EC.1.1.3.22) was evaluated according to the method described by Ferraz Filha *et al*. (2006) with light modifications. The percentage of inhibition is determined by kinetic measurement with 295 nm associated with the formation of uric acid (Owen and Johns, 1999).The reactional mixture consists of 50 µl of extract to the final concentration of 200 µg/mL, 150 µL of phosphate buffer (pH 7.5, 1/15 M) and 50 µL of enzyme solution (0.28 U/mL prepared in the buffer).After pre-incubation of the mixture with 25ºC during 1 min, the reaction is initiated by adding 250 µL of a solution of substrate (0.6 mm) and the absorption is measured during 120 S. A control is prepared without extract. The analyses are carried out by triplet. Allopurinol, an inhibitor of reference is used like positive control. The percentage of inhibition of the XO is calculated as follows:

$$1(\%)\frac{vo - v}{vo} \times 100$$

%:percentage of inhibition of the XO. Vo: variation of the absorption of the test without the plant extract. V: variation of the absorption of the test with the plant extract.

Phytochemistry assessment methods:

The total phenolic content: of each fruit extract were determined as described by Lamien-Meda *et al*. (2008). The diluted aqueous solution of each extract (0.5 mL) was mixed with FolinCiocalteu reagent (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/L in water, 2 mL) was added. After 2 h of incubation, the absorbances were measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0-100 mg/L). The straight line equation is y = 0.0095x, with R^2 = 0.9966.The results were expressed as mg of gallic acid equivalents (GAE)/100 g of plant weight.

The total flavonoid content was estimated according to Lamien-Meda *et al*. (2008). A diluted methanolic solution (2 mL) of each fruit extract was mixed with a solution (2 mL) of aluminiumtrichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 mL) and plant extract (2 mL) without $AlCl_3$. Quercetin(0-50 mg/L) was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100 g of plant weight. The straight line equation is y = 0.0249x, with R^2 = 0.9943:

The total flavonol content was estimated according to the method of Almaraz-Abarca *et al*. (2007). In each tube, it is mixed 750 µL of extract (0.1 mg/mL) prepared in ethanol and 750 µL of an aqueous solution of $AlCl_3$ (20%). For each extract, the test is carried out in triple. A blank sample is carried out by the mixture of extract (750 µL) and ethanol (750 µL). The optical density is read after 10 mn of incubation to the wavelength of 425 nm. Quercetin (0-50 mg/L) was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100 mg of plant weight.

Anti-nutritionalevaluation methods:

Oxalate content determination: Briefly, thdetermination of Oxalate content was previously described by Oke (1966). Two (2) g of the sample was digested with 10 mL

of HCl (6M) for one hour and made up to 250 mL in a volumetric flask. The pH of the filtrate was adjusted with NH_4OH (28%) until the color of solution changed from salmon pink color to a faint yellow color. Thereafter, the filtrate was treated with 10 mL of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension is now centrifuged at 2500 rpm, after which the supernatant was decanted and precipitate completely dissolved in 10 mL of 20% (v/v) H_2SO_4. The total filtrate resulting from the dissolution in H_2SO_4 is made up to 300 mL. An aliquot of 125 mL of the filtrate was heated until near boiling point and then titrated against 0.05 M of standardized $KMnO_4$ solution to a faint pink color which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titer value. The overall redox reaction is.

$$2MnO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2$$

The total tannin content quantification: The total tannin content was proportioned by using the reference method of European commission (2000). 200 μL of extract was mixed with 1ml of distilled water, 200 μL of ferric ammonium citrate(3.5 g/L) prepared freshly and 200 μL of ammoniac (20%). The solution absorption is measured at 525 nm after 10 mnsof incubation against a blank (200 μL extract + 1200 μL distilled water).

Tannic acid (0-150 mg/L) was used as reference compound to produce the standard curve, and the results were expressed as mg of tannic acid equivalent (TE)/100 mg of plant weight.

Statistical analysis: Results were expressed as the mean ±standard deviation of triplicate analysis. Statistical comparisons were performed using Analysis of Variance (ANOVA) of XLSTAT Pro. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Extraction yields: The various extractions gave the yields between 7.1 and 17.32. The yield of the aque decoction extract showed the most significant values: 17.32, 9.64, 15.4 and 13.08, respectively for *A. dubius*, *A. graecizans*, *A. hybridus* and *A. hybridus*. This translated the richness of the four species in water soluble polar compounds. The aqueous decoction extract is particularly interesting because it corresponds to the method of traditional use.

Nutritional factors: The proteins, β-carotene and lycopene content of the four species are consigned 1. The contents of proteins in the various extracts are included between 24.42±4.17 and 51.56±3.37 mg BSA Equivalent/100 mg. The decoction allowed the best protein extractions. A. hybridus shows a more significant content of proteins with 56±3.37 mg BSA Equivalent/100 mg for the aqueous extract. The amount of *A. dubius* and *A. graecizans* are appreciably close as well in the Aqueous Extract (AE) as in Methanolic Extract (ME).

A protein concentrate obtained from *A. hybridus* showed a content of protein of 35.1 mg/100 mg of matter (Aletor and Adeogun, 1995). Although the adopted method is different from ours, this result is comparable with those of ME of *A. hybridus* which is 37.89 mg BSA Equivalent for 100 mg of extract.

The contents of β-carotene are of 13.94±0.79, 5.72±0.42, 0.98±0.21, 3.05±0.53 mg/ 100 g of dries matter, respectively for *A. dubius*, *A. graecizans*, *A. hybridus* and *A. viridis.*

Studies carried out (Rajyalakshmi *et al.*, 2001; Singh and Sehgal., 2001) reported that the leaves of amaranth contain between 2.3 and 14.7 mg of β-carotene /100 g of dry matter. The content of our plants is in the tendency indicated except for *A. hybridus* which contains less.The regular consumption of these amaranths thus takes part to avoid the deficiencies in vitamin A.

The food plants contribute to approximately more than 80% of source of dietetic vitamin A in the countries in the process of development (Bhaskarachary *et al*., 1995). The plants molecules, such as carotenoids, present effective antioxidant properties. A food rich in β-Carotene would make it possible to decrease the rate of vitamin Adeficiency as well as the synthetic supplementation of vitamin A (Gopalan, 1992).

The lycopene contentare of 0.22±0.05, 4.28±0.98, 0.27±0.092, 0.15±0.06 mg/100 g of dries matter, respectively for *A. dubius, A. graecizans, A. hybridus* and *A. viridis*. The lycopene content of our four species in fresh mattervaries between 0.25 and 7.27 mg/kg. These amountsare very weak compared to other vegetables as the tomato (*Lycopersiconesculentum)* which is of 145.50 mg/kg fresh matter (Basuny *et al*., 2009)

Mineral composition: The content of minerals is higher at followed by*Amaranthus hybridus* and *Amaranthus viridis*. *Amar amaranthusdubius*, *Anthus graecizans* is lowest in minerals.

The magnesium amount of these species is comparable with those reported by Ladan *et al*. (1996), Antia *et al*. (2006) and Hassan and Umar (2006) in some green vegetables. It is a significant biogenic salt in connection with the cardiac ischemic diseases and the metabolism of calcium in bones (Ishida *et al*., 2000).

Iron is essential for the formation of hemoglobin, normal operation of the central nervous system and in the oxidation of the carbohydrates, proteins and greases (Adeyeye and Otokili, 1999).

The zinc content (3.01 mg/100 g for *A. hybridus*) is comparable with the majority of the values reported for

Table 1: Protein, β-carotene and lycopene content

Species	*A. dubius*	*A. graecizans*	*A. hybridus*	*A. viridis*
Protein mgAE	32.44±2.93^{d}	36.11±4.88^{c}	51.55±3.37^{a}	45.72±4.88^{b}
BSAE/100mg of ExtractME	24.41±4.17^{b}	23.86±2.93^{b}	37.88±5.67^{a}	13.97±1.73^{c}
β- Carotene mg/100g Dries Matter	13.94±0.79^{a}	5.72±0.42^{b}	0.88±0.21^{d}3.	05±0.53^{c}
Lycopene mg/100g Dries Matter	0.22±0.05^{b}	4.28±0.98^{a}	0.27±0.09^{b}	0.15±0.06^{c}

Data are mean±SEM (n = 3); AE: aqueous extract; ME: methanolic extract; Values showing the same letter are not significantly different (p<0.05) from one other in the same line

Table 2:Contents of minerals mg/100g in dries matter

Espèces	*A. dubius*	*A. graecizans*	*A. hybridus*	*A. viridis*
Na	15.32±0.11^{d}	6.92±0.07^{c}	6.17±1.25^{b}	27.2±0.08^{a}
K	64.09±0.06^{a}	5.02±0.08^{d}	52.20±2.60^{b}	47.15±0.12^{c}
Ca	55.71±0.01^{a}	2.55±0.07^{d}	50.29±1.32^{b}	37.86±0.10^{c}
Mg	186.18±0.08^{b}	2.64±0.08^{d}	209.10±2.79^{a}	111.09±0.12^{c}
P	53.81±0.12^{a}	7.95±0.09^{d}	37.66±1.10^{b}	23.11±2.11^{c}
Fe	8.24±0.52^{b}	0.21±0.04^{d}	11.25±0.09^{a}	4.56±0.11^{c}
Mn	1.31±0.09^{a}	0.16±0.06^{d}	1.10±0.08^{b}	0.41±0.09^{c}
Zn	5.86±0.10^{b}	0.25±0.01^{d}	3.01±0.74^{c}	6.79±0.06^{a}
Cu	11.67±0.16^{a}	0.39±0.07^{c}	11.17±1.45^{a}	9.10±0.01^{b}

Data are mean±SEM (n = 3); Values showing the same letter are not significantly different (p<0.05) from one other in the same line

Table 3: Antioxidant activity and inhibiting capacity of the xanthine oxidase

Antioxidantactivity	XO Tested at 200 ug/mL (Percentage)	β Carotene assay (EC_{50} mg/mL)	DPPH (µmol AEAC/g extract)
A. dubius	11.58±0.04^{a}	1.26±0.46^{b}	49.5±2.15^{c}
A. graecizans	6.61±0.04^{c}	1.43±0.11^{b}	62.82±0.76^{b}
A. hybridus	12.05±0.05^{a}	1.50±0.10^{b}	66.99±1.65^{a}
A. viridis	8.35±0.02^{b}	2.73±0.21^{a}	33.67±2.06^{c}
Allopurinol(at 100 µg/mL)	96.38±0.60	-	-
α-Tocopherol (Control)	-	0.49±0.0	1
Quercetin			13760±0.26

Data are mean±SEM (n = 3); Xo: xanthine oxidase inhibition; Values showing the same letter are not significantly different (p<0.05) from one other in the same columns

Table 4: Chemical composition

Species	Extract	Phenolics mg GAE/100 mg of extract	Flavonoids mgQE/100 mg of extract	Flavonols mgQE/100 mg of extract
A. dubius	AE	10.00±0,18	^{c}1.33±0.26^{d}	0.68±0.07^{b}
	ME	12.08 ±0.31^{c}	5.50±0.37^{d}	0.95±0.03^{a}
A. graecizans	AE	15.34±0.93^{b}	1.58±0.28^{b}	0.26±0.04^{d}
	ME	12.37±0.47^{b}	5.11±0.5^{c}	0.84±0.01^{b}
A. hybridus	AE	22.42±0.3^{a}	12.73±1.61^{a}	1.46±0.05^{a}
	ME	17.63±0.38^{a}	6.69±0.19^{b}	0.12±0.01^{d}
A. viridis	AE	8.46±0.17^{d}	1.51±0.3^{c}	0.42±0.03^{c}
	ME	11.78±0.1^{d}	6.93±0.22^{a}	0.39±0.02^{c}

Data are mean±SEM (n = 3); AE: aqueous extract; ME: methanolic extract; Values showing the same letter are not significantly different (p<0.05) from one other in the same columns

Table 5: Contents of tannin and oxalate

Espèces		*A. dubius*	*A. graecizans*	*A. hybridus*	*A. viridis*
Tannins	AE	1.74±0.03^{a}	2.27±0.04^{a}	3.00±0.03^{a}	1.85±0.08^{a}
mg TAE/100 mg of extract	ME	1.50±0.07^{a}	1.25±0.10^{a}	3.68±0.27^{a}	1.32±0.11^{a}
Oxalate mg/100 g of dries matter		595.83±15.87^{a}	330±27.5^{c}	504.16±15.87^{b}	247.5±27.5d

Data are mean±SEM (n = 3); AE: aqueous extract; ME: methanolic extract; Values showing the same letters are not significantly different (p<0.05) from one other in the same line

vegetables in the literature (Ibrahim *et al*., 2001; Hassan and Umar, 2006). Zinc is implied in the normal operation of the immune system.

The ratio of Na/K in the body is of great importance for the prevention of hypertension. A ratio of Na/K lower than1 is recommended (FND, 2002).Consequently, the consumption of *A. dubius*, *A. hybridus* and *A. viridis* is advised with the patients suffering from hypertension.

Calcium and phosphorus are associated at the growth and the maintenance of the bones, the teeth and the muscles (Dosunmu, 1997; Turan *et al*., 2003). For a good intestinal absorption of Ca and P, the ratio of Ca/P should be close to 1 (Guil-Guerrero *et al*., 1998).*A. dubius* answers this requirement and would be certainly a good source of Calcium and especially of Phosphor for the body.

Functional activity: The antioxidant potential of the extracts would be responsible for the prevention of the cardiovascular and neurodegenerative diseases (Heim *et al*., 2002) and of the bones diseases (Govindarajan *et al*., 2005). It would prevent also cancers (Kawanishi *et al*., 2001).

The antioxidant activity of the AE of *A. dubius*, *A. graecizans*, *A. hybridus* and of *A. viridis* was evaluated with β-carotene bleaching assay and DPPH radical scavenge.The evaluation of the antioxidant activity of the extracts of the four species showed an activity dose dependent. Their CE50 are respectively 1.26, 1.43, 1.50, and 2.73 mg/mL for *A. dubius, A. graecizans, A. hybridus* and *A. viridis*.The antioxidant activity of α-tocopherol (EC50) used as control was of 0.49±0.01 mg/mL.

Our extracts reduce the discoloration of the β - carotene. In more we notice that *A. dubius, A. graecizans* and *A. hybridus* showed the same anti-oxidizing capacities and 3 times less than control. *A. viridis* was shown the least active. This would be due to the phenolic compounds and especially to the β-carotene whose *A. viridis* contains less.

The DPPH scavenging activities of the extracts, expressed as a μmol AEAC/g extract value, ranged from 33.67±2.06 at 66.99±1.65. These results showed a weak activity compared to quercetin (13760±0.26) used as control.

These plants are not a good source of natural antioxidant. Studies carried out by Ozsoy *et al*. (2009) could show that other species of the same family as *Amaranthus lividus*stems with leaves and flowers seem to be good sources of natural antioxidants.

The inhibition percentages of the XO go from 6.619±0.04% for *A. graecizans* to 24.35±0.02% for *A. viridis.* Compared with the inhibiting capacity of the allopurinol 93.2% at 100 μg/mL on XO, these extracts show a weak activity.

Phytochemistry: The extraction and the proportioning of polyphenols are a current practice in the evaluation of medicinal plants. The most significant content of polyphenol is found in the aqueous decoction extract of *A. hybridus* (12.73±1.61 mg GAE/100 mg of extract. The contents of phenolic of the four species are different and decrease respectively from *A. dubius*, *A. graecizans, A. hybridus* at *A. viridis*. Water extracts the compounds better than the methanol. It is also the food mode of use of these amaranths, as in sauces.

The most significant contents of flavonoids were observed in the AE followed by the ME.The proportion of flavonols is relatively significant. These flavonols including rutin would then have a dominating role in the action of the flavonoids.

Polyphenols, flavonoids, tannins and saponosides (Owen and Johns, 1999) would be potentials inhibiting of the XO. The low contents of polyphenols in these species would explain their weak inhibiting activity of the xanthine oxidase.

Anti-nutritive factors: The contents of tannin vary between 3.68 (ME of *A. hybridus*) and 1.25 mg TAE/100 mg (ME of *A. graecizans*).

The contents of tannin for 100 mg of dry vegetable material were evaluated. These rates vary between 0.024 (ME of *A. viridis*) and 0.066 mg/100 mg for *A. hybridus*. These values are definitely comparable with those reported by Fasuyi (2007) which are of 0.068 mg/100 mg of dried leaves for *A. hybridus*.

The proportioning of the tannin is interesting in human nutrition because they constitute an antinutritionnel factor. The high percentages of tannin would have a negative influence on the digestibility of proteins and the contribution in amino acids (Fasuyi, 2007).

Oxalate content: The oxalate content found varies between 247.50±27.50 and 595.83±15.87 mg/100 g of dries matter. *A. dubius* has the high content of oxalate. By taking account of the water content, *A. hybridus* contains approximately 84.02 mg/100 g of fresh matter. The oxalate content of *A. hybridus* corroborates work of Savage (2000) which found 90.9 mg/100 g fresh matter in the leaves of *A. hybridus*.

dose dependent. Their CE50 are respectively 1.26, 1.43, 1.50, and 2.73 mg/mL for *A. dubius*, *A. graecizans*, *A. hybridus* and *A. viridis*.The antioxidant activity of α-tocopherol (EC50) used as control was of 0.49±0.01 mg/mL.

Our extracts reduce the discoloration of the β-carotene. In more we notice that *A. dubius, A. graecizans* and *A. hybridus* showed the same anti-oxidizing capacities and 3 times less than control. *A. viridis* was shown the least active. This would be due to the phenolic compounds and especially to the β-carotene whose *A. viridis* contains less.

The DPPH scavenging activities of the extracts, expressed as aμmol AEAC/g extract value, ranged from 33.67±2.06 at 66.99±1.65. These results showed a weak activity compared to quercetin (13760±0.26) used as control.

CONCLUSION

This study, reveals that *Amara dubius* Mart. ExThell *Amaranthus graecizans* L. *Amaranthus hybridus* L and *A. viridis* L. appreciably present the same chemical profile but with different amounts.

A. hybridus and *A. dubius* content the high level of mineral and proteins. The amounts of protein and micro elements of species explain their uses especially in the infantile nutrition as food supplement.

These four species usually used in food linked of the contents of natural substances with therapeutic virtues such as β-carotene, proteins and minerals. These four species could thus contribute considerably to fight pathologies of oxidative stress more and more running in developing countries. The everyday consumption of these amaranths could have a positive impact on pathologies like cancers, diabetes, hypertension and neurodegenerative diseases.

It is also advised a moderate consumption of these species forthe people predisposed of the renal calculi, especially *A. hybridus* and *A. dubius*.

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