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Research Article

Acethylcholine esterase, Lypoxygenase inhibition of four Amaranthaceae and their content of vitamine (A,E,K), betalain.

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Abstract

The optimal food supply of a certain number of substances which one finds in the plants, the animals and the minerals are one of the major prerequisites to the maintenance of health. The percentage of Ache and Lox inhibition at 1mg/l was lower than 50%. We note however that *A. hybridus* is most active on the acetyl choline esterase with 26.70 ± 2.04% of inhibition for the aqueous extract. In comparison, quercetin at the concentration of 25ug/ml gave 54.11 ± 0.66% of inhibition. Amaranth contain β-carotene between 2.3 and 14.7 mg/100 g of dry matter. This is in the trend indicated above safe for *Amaranthus graecizans* which contains some less. The contents α-tocopherol vary 0.133mg/100g for *Amaranthus graecizans* at 0.981mg/100g for *Amaranthus hybridus. Amaranthus dubius* is less rich in vitamin k1 (0.015mg/100g) contrary to Amaranthus viridis which contains some more (0.084mg/100g). Compared to the recommended daily intake (0.075mg), the amaranths are very rich in vitamin C. The contents of fibers for *A. dubius*, *A. graecizans*, *A. hybridus* and *A. viridis* are respectively of 7.09±2.04, 12.34±1.89, 6.25±1.33 and 4.8± 1.75% of dry matter.

Keywords: Amaranthaceae -Acethylcholine esterase -Lypoxygenase - vitamin A,E,K - Fiber

Introduction

Oxidation induced by reactive oxygen species (ROS) can damage membranes, lipids, lipoproteins, and induce DNA mutation. This type of cell or tissue injuries has been associated with aging, atherosclerosis, carcinogenesis, cardiovascular- and Alzheimer's diseases. Preventing or minimizing these oxidation-related diseases may involve the use antioxidant substances that scavenge and eradicate ROS, namely the superoxide- (O2•-), hydroxyl- (HO•), peroxyl- (ROO•), and nitric oxide radicals (NO•) (TEPE and al, Duan and al.). In addition to the beneficial effects of the antioxidants in human health, some of them are also used in food industry as preservatives for preventing or delaying the oxidation process.

Plants have enough antioxidant properties and one of the most important resources of human foods and medicines.

Rapidly increasing knowledge on nutrition, medicine, and plant biotechnology has dramatically changed the concepts about food, health and agriculture, and brought in a revolution on them. Nutritional therapy and phytotherapy have emerged as new concepts and healing systems have quickly and widely spread in recent years. Strong recommendations for consumption of nutraceuticals, natural plant foods, and the use of nutritional therapy and phytotherapy have become progressively popular to improve health, and to prevent and treat diseases. With these trends, improving the dietary nutritional values of fruits, vegetables and other crops or even bioactive components in folk herbals has become targets of the blooming plant biotechnology industry. For this reason we were interested under investigation of the 4 food plants (*Amaranthus dubius*,

Amaranthus graecizans, Amaranthus hybridus and *Amaranthus viridis*) of Burkina.

Materials and Methods

Plant sample collection and identification

The leaves of the four plants were harvested from kitchen garden and farm located in Ouagadougou, central plate, Burkina Faso. Plants were also botanically identified by Prof. Millogo from the Plant Biology Department of the University of Ouagadougou and voucher specimen deposited at the herbarium of, University of Ouagadougou. The plant materials were cleaned, rinsed with deionized water, and allowed to evaporate at room temperature.

Determination of AChE activity

Enzyme activity was measured by the method of Ellman et al., (1961). Enzyme activity reaction mixture (200 µl) consisted of 160 µl of 50 mM Tris HCl buffer, pH 7.4, with/without plant extract followed by the addition of 10 µl enzyme (40-60 µg protein) from fresh chicken liver homogenate in 96-well plates. The contents were mixed and preincubated for 10 min at 250°C. Plates were pre-read at 412 nm using Synergy HT BioTek (USA) plate reader. The reaction was initiated by the addition of 10 µl of 1 mM DTNB and 3 mM substrate acetylthiocholine iodide. After 15 min incubation, absorbance was measured at 412 nm within 4-7 min. Control experiments were carried out to correct for non-enzymatic hydrolysis by adding enzyme after the addition of DTNB. Absorbance values were subtracted from the control and data presented as percent inhibition of enzyme activity. All experiments were carried out with their respective controls in triplicate.

In-vitro lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed previously (Khan et al., 2009; Khan et al., 2011b). Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from Sigma (St. Loius, MO) and were used without further purification. All other chemicals were of analytical grade. 160 ml of sodium phosphate buffer, 0.1 mM (pH 7.0), 10 ml of the sample solution and 20 ml of lipoxygenase solution were mixed and incubated for 5 min at 25°C. The reaction was initiated by the addition of 10 µl linoleic acid solution substrate and the absorption change with the formation of (9Z, 11E)-13S-13-hydroperoxyoctadeca-9, and 11-dienoate followed for 10 min. The test sample and the control were dissolved in 50% ethanol. All the reactions were performed in triplicate.

Vitamine A,E, K assessment

The protein of sample (0.1g) is eliminated by addition of 500 ul of pyrogallol 1% (antioxydant) in absolute ethanol and is saponified by 200 µl of a KOH solution (3.6 M) in a sonicator, 30 min at 65°C and under nitrogen atmosphere. The samples are then plugged by 500 µl of NaH2PO4 (0.2 M, pH 7.8) and a double extraction with hexane is carried out. The separation and the quantification of the vitamins are carried out by HPLC (column DiscoveryTM C8, 25 cm × 4.6 mm, 5 µm) (with a mobile phase methanol-acétonitrile-dicholorométhane 10/70/20, v/v) and a spectrophotometric detection with the wavelengths of 450 nm for B-carotene (time of retention 6 at 7mn), 248 nm for the vitamin K1 (time of retention 4 at 5mn) and 294 nm for the vitamin E (time of retention 3 at 4 mn).

Fiber

The rough fibers gather cellulose, some hemicelluloses and lignin. The contents of rough fibers of the samples are determined by the method of AFSSA (2002). For that, 1g of sample (M) is brought to a boil in 50 ml of sulphuric acid (0.25N) and then in 50 ml of NaOH (0.31 M) during 1:00 (30 min*2). The residue obtained is dried at 105°C during 8:00 (M1) then incinerated at 550°C during 3:00 (M2). The content of rough fibers (RF) is expressed as a percentage dry matter (ms) by the formula: RF (%) = (M1-M2) /M *100

Results and Discussion

Acethylcholine esterase inhibition activity

The results of the inhibition of Ache are presented in table 1. In this inhibition of the Ache, at the concentration of 1mg/ml, none methanolic and aqueous extract gave 50% inhibition. We note however that A. hybridus is most active with 26.70 ± 2.04% of inhibition for the aqueous extract. The galanthamine which is an inhibitor of the Ache gave 98.28 ± 1.20% of inhibition at 10 µg/ml.

Aqueous and methanolic extract of the four plants presented moderated activities in the inhibition of the Ache. The alkaloids, the terpenoides, glucosides and coumarins are the principal families of compounds recognized as inhibitors of the Ache (Lee and al., 2004). The alkaloids and the terpenoides were not highlighted

in the plants. The flavonoïdes can also contribute to this inhibition (Lopez and al., 2002). Our plants contain of it very little and that could explain the weak inhibition of the Ache.

Lipoxygenase inhibition

Table 2 show the results got by the inhibition of the LOX by the various extracts. With the concentration of 1 mg/ml we did not obtain 50% of inhibition. The aqueous extracts of A. dubius shown the high inhibition. This result shows a weak inhibiting activity of the extracts compared to inhibition of the quercetin (54.11 ± 0.66%) at 25ug/ml, which is an inhibitor well-known and easily available of the 15-lipoxygénase (Wangensteen and al., 2004).

These compounds have a significant role in the physiopathology of several inflammatory diseases especially allergic (Bhattacharjee, 2007), and particularly the 15-lipoxygénase are suggested playing a part in the development of atherosclerosis, probably of with its capacity to peroxidize the low density lipoproteins (LDL) (Cyrus and al., 2001). In the present study all the four plants did not show good inhibiting activities of the 15-LOX of soybean at the concentration of 1 mg/ml using linoleic acid like substrate, which translates the low therapeutic potential that these plants can have in the treatment of certain inflammatory diseases.

Vitamins

Vitamins are organic substances, necessary in small quantity to the metabolism of a living organism, which cannot be synthesized in sufficient quantity. The vitamins are essential complements to the vital exchanges. The contents of vitamins are consigned in the table 3:

β-carotene

Studies carried out (Rajyalakshmi and al., 2001; Singh and al., 2001) reported that the sheets of amaranth contained between 2.3 and 14.7 mg/100 g of dry matter. The content β-carotene of our plants is in the trend indicated above safe for *A. graecizans* which contains some less. The regular consumption of these amaranths thus takes part to avoid vitamin A deficiencies. The food plants contribute to approximately more than 80% of source of dietetic vitamin A in the countries in the process of development (Bhaskarachary and al., 1995). The phytomolecules, such as carotenoids, present effective antioxydant properties. Also β-carotene could explain the use of the amaranths in the cases of pains, fever, burns.

α-tocopherol

The contents of α-tocopherol vary to 0.133mg/100g for *A. graecizans* at 0.981mg/100g for *A. hybridus*. The amaranths are less rich in vitamin E compared to *Moringa Oleifera* which contains 77mg/100g of dry matter. (BUSANI and al., 2011).

Phylloquinone

A.dubius is less rich in vitamin k1 (0.015mg/100g) contrary to *A. viridis* which contains some more (0.084mg/100g). The vitamins K form a group of liposoluble vitamins necessary for the post-traductionnelles modifications of certain proteins intervening primarily in blood coagulation but also in the metabolism of the bones. They come primarily from the food in particular of green vegetable foods, because related to the chloroplasts. They support the synthesis of blood coagulation factors, the fixing of calcium by the bones, the flexibility of the arteries and the good state of the blood-vessels in general, of the tendons, cartilages. The consumption of *A. viridis* and *A. hybridus* could meet the daily needs recommended.

Ascorbic acid

Compared to the recommended daily intake (0.075mg), the amaranths are very rich in vitamin C. the vitamin C is a water-soluble vitamin sensitive to heat and the light playing a significant role in human metabolism being and of many other mammals. It is necessary in the synthesis of collagen and the red globules. She also plays a part in the metabolism of iron as a promoter of her absorption. Very fragile in solution, it is destroyed in contact with the air (oxydation) or under the exposure to the light (by action of the ultraviolet rays). The heat of cooking (between 60 and 75°C) of food destroys the vitamin C (Vojdani, 2000). Because of the mode of preparation and brittleness of this vitamin, these species as consumed would not take part in the vitamin C contribution.

Fiber

The contents of fiber for *A. dubius, A. graecizans, A. hybridus* and *A. viridis* are respectively of 7.09±2.04, 12.34±1.89, 6.25±1.33 and 4.8± 1.75% of dry matter.

Table 1: Acethylcholine esterase inhibition activity

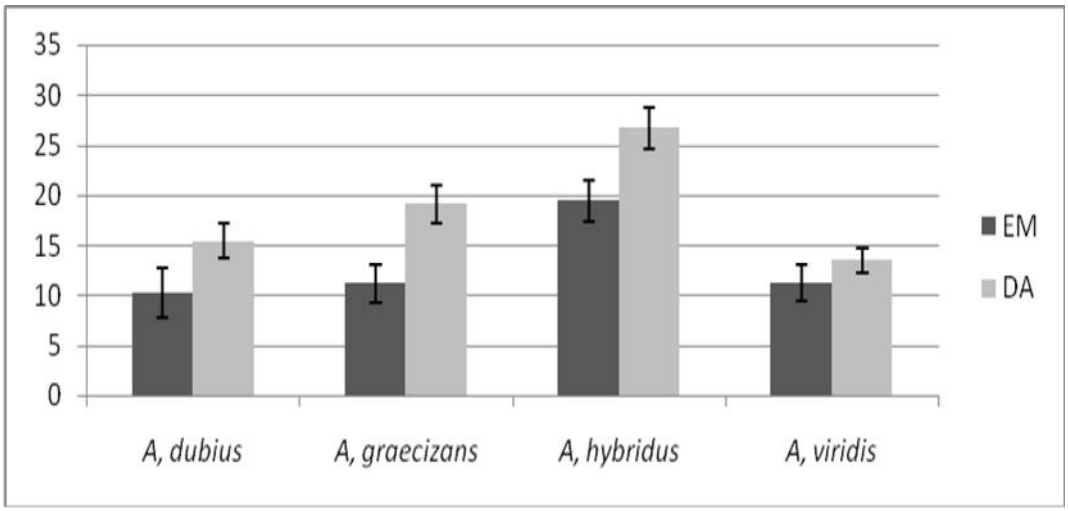

Table 2: Lipoxygenase inhibition

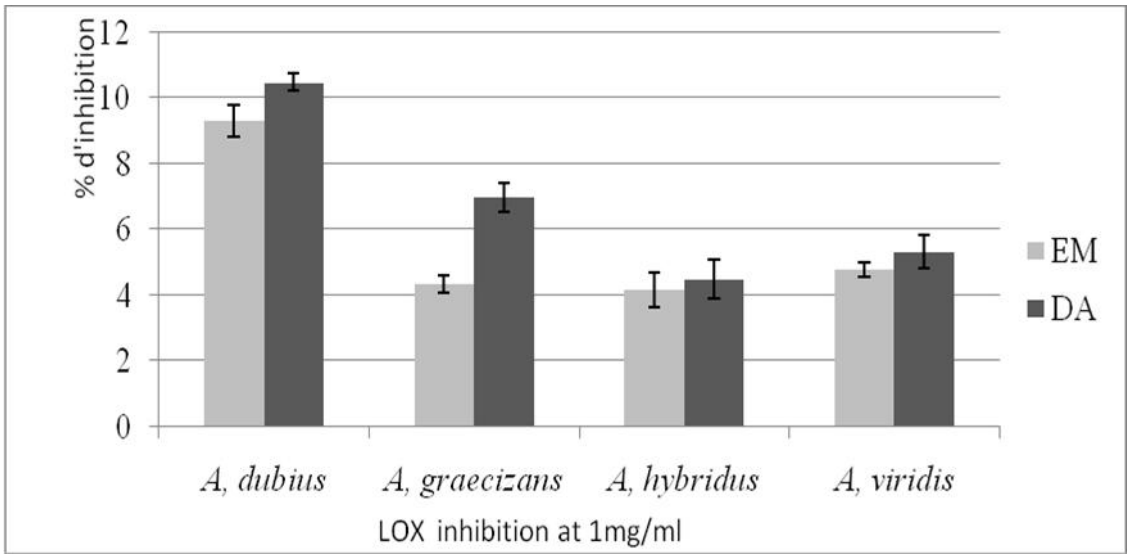

Table 3. The contents of vitamins

species	*A. dubius*	*A. graecizans*	*A. hybridus*	*A. viridis*	RDI(mg)
β-carotene	6,453±0.536	1,638±0.197	5,480±0.198	3.247±0.222	0.6
α-tocopherol	0,252±0.01	0.133±0.041	0,981±0.015	0,785±0.021	10
Phylloquinone	0.015±0.003	0.024±0.003	0,069±0.005	0.084±0.006	0.08
Ascorbique acid	19.458±1.04	14.784±0.978	26.154±1.67	18.849±1.04	0.075

The results got at *A. dubius, A. hybridus and A. viridis* are not statistically different ($p<0.005$).

A. graecizans has the strongest content. Fibers also support satiety, slow down the absorption of glucids and cholesterol. The fibers are an asset for better balancing the food supplies. Then, to recommend a balanced food sufficiently rich in fiber is part of the actions of education for health (AFSSA, 2002). The beneficial properties of fiber are related to their water holding capacity and on their fermentation by the bacteria of the colon. Lastly, even if uncertainties remain, the protective effect of the fruit and vegetables (and overall of rich foods in fiber) with respect to digestive cancers is currently regarded as probable (Funds world of research against cancer, 2007). This results watch that the amaranths are thus plants food sources of fibers and that would justify their use as laxative food.

Conclusion

The general objective of this work was to contribute to the valorization of the medicinal and food plant of Burkina Faso, through the evaluation nutraceutic potentialities of *Amaranthus dubiusMart. Ex Thell, Amaranthus graecizans L., Amaranthus hybridus L.* and *Amaranthus viridis L.* The great food use of these four species is such as one needs a perfect control of all the nutritional factors (vitamins and oligo elements) and antinutritionnels factors (oxalate, tannins, phytic acid, lectines) and their evolution according to the mode of preparation. It is interesting to know that these four species usually used in food contains natural substances with therapeutic virtues such as vitamins and fibers even if they did not show an interesting enzyme inhibiting activity. Thus, these four species could contribute to a significant degree to improve the food quality needs for our populations.

Acknowledgment

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