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Full Length Research Paper

Betacyanins are the most relevant antioxidant molecules of *Amaranthus spinosus* and *Boerhavia erecta*

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Ethnobotanical investigations have shown that the redder *Amaranthus spinosus (A. spinosus)* and *Boerhavia erecta* (*B. erecta*) specimens are, the more they are used for traditional medicinal purposes. This work aimed to elucidate the role of the betalain pigments in the bioactivity of the Caryophyllales species. Histochemical and biochemical studies on the two species (*A. spinosus* L. and *B. erecta* L) indicate that the synthesis and subsequent storage of betalain pigments in the stems, leaves and root epidermis or in the cortical parenchyma are induced by stress (biotic or abiotic). Radical scavenging activity assays using 2, 2'-Azinobis, 3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) free radicals showed that the betalain-containing fractions of these plants have the highest activity. We suggest that reactive oxygen species (ROS), of either biotic or abiotic origin, are signals that induce the biosynthesis of betalains, which act as ROS scavengers. The formation of these compounds has been observed and appreciated by traditional healers as evidence of the usefulness of this drug.

Key words: Betalains, phenolic, oxidative stress, antioxidants, *Amaranthus spinosus, Boerhavia erecta*.

INTRODUCTION

Oxidation is an essential biological process for energy production in many living organisms. Since some years, growing evidence has been accumulated indicating the involvement of reactive oxygen species (ROS) in the pathogenesis of many diseases. Although, the human body possesses an inherent antioxidant defence system to protect against oxidative damage; it is unable to entirely prevent the damage caused by excess ROS production. Indeed, excessive ROS produced *in vivo* during some oxidative reactions (Bloknina et al., 2003), are not only strongly associated with lipid peroxidation, but also involved in the development of a variety of physiological conditions including cellular aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes and neurodegeneration (Halliwell and Gutteridge, 1999). Recently, there have been increasing reports that some medicinal or edible plants may afford protection and/or treatment of some chronic diseases. Generally, these beneficial effects are attributed to their antioxidant constituents, including polyphenols, vitamins, carotenoids, flavonoids, catechins, anthocyanin or betalains, etc. (Zhao et al., 2005).

Caryophyllales order constitutes a singular example in which betalains, a class of secondary metabolites, replace anthocyanins in the flowers and fruits of most families of these angiosperms (Mabry, 1980). Unexpectedly, these pigments are also found in some higher fungi

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Abbreviations: ABTS, 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid; **ROS,** reactive oxygen species; **FCR,** Folin-Ciocalteu reagent; **H_2O_2,** hydrogen peroxide; **RSC_{50},** radical scavenging capacity 50%.

(Strack et al., 2003). Whereas the anthocyanin-analogous functions of betalains as pigments of flowers and fruits are obvious (attractors for pollination and seed spreading) (Vogt et al., 1999; Chalker-Scott, 1999; Stafford, 1994), their role in the fungi, stems and underground roots of plants remains obscure.

Betalains are water-soluble nitrogen-containing pigments (or chromoalkaloids), which include the red-violet betacyanins and the yellow betaxanthins. They are immonium conjugates of betalamic acid with cyclodopa and amino acids or amines, respectively (Strack et al., 2003).

Amaranthus species (Amaranthaceae) and *Boerhavia* species (Nyctaginaceae) are used in tropical and subtropical countries for human nutrition both as vegetables (*Amaranthus* and *Boerhavia*) and grains (*Amaranthus*), but also as animal feed (Berghofer and Schoenlechner, 2002; Miralles et al., 1988). Furthermore, members of both genera are popular medicinal plants to treat several ailments such as malaria, hepatic disorders, jaundice, and scanty urine or to cure wounds (Berghofer and Schoenlechner, 2002; Samy et al., 1999; Srivastava et al., 1998).

Stem bark extracts of *Boerhavia erecta* L. (erect spiderling) and *Amaranthus spinosus* L. (spiny amaranth), two wild growing weed plants used in African traditional medicine, were characterized with respect to their phenolic profile including the betalains. While the main betalains in *A. spinosus* were identified as amaranthine and isoamaranthine, and the major betacyanins in *B. erecta* were betanin, isobetanin together with neobetanin. Extracts of *A. spinosus* were found to contain hydroxycinnamates, quercetin and kaempferol glycosides, whereas catechins, procyanidins and quercetin, kaempferol and isorhamnetin glycosides were detected in *B. erecta.* Ethnobotanical investigations (Nacoulma-Ouedraogo, 1996) demonstrated that the redder these specimens are, the more they are harvested for traditional medicinal purpose. However, little information on the antioxidant activity of these two species (betalains and phenolic compounds) is available. Based on previous results, the polyphenols and betalains antioxidant activities were investigated in order to get more insight on the evolutionary importance of betalains for these tropical species submitted to huge biotic and abiotic stress.

MATERIALS AND METHODS

Reagents

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, betanin, amaranthin, 2, 2'-Azinobis, 3-ethylbenzothiazoline-6-sulphonic acid (ABTS), horseradish peroxidase, chlorhydric acid, tannic acid, and hydrogen peroxide were purchased from Sigma-Aldrich (Chemie, Steinheim, Germany). Ethyl acetate, acetate anhydride, sodium carbonate, and trifluoroacetic acid (TFA) were purchased from LABOSI (Paris, France).

Acetic acid was purchased from SDS (Peyin, France); 28% ammonia, ethanol and *n*-hexane were purchased from VWR (International GmbH, Darmstadt, Germany); methanol was purchased from FLUKA Chemie (Buchs, Switzerland). Microscope with camera (Olympus CH30, OlympusSC35 type12), Soxhlet apparatus (BARNSTEAD ELECTROTHERMAL), Spectrophotometer (CECIL CE 2041), Lyophilisator (Telstar Cryodos 50), Rotavapor (Buchi 461) and Centrifuge (ALC, 4206) were used.

Plant

Mature red stems of *A. spinosus* L. and *B. erecta* L. were collected in December in the old experimental garden area of the University of Ouagadougou. Voucher specimens (Hilou A1 and Hilou A2) were identified by Professor Millogo-Rasolodimby Jeanne (botanist) and deposited at the herbarium at the University of Ouagadougou. The barks were removed from the stems with a knife, dried in the laboratory at 30°C for 36 h, and pulverized in a laboratory mortar. These ground materials were stored out of light and dampness.

Histological and phytochemical analyses

Histological analysis can be used to study the location of plant's metabolites within its tissues. Histological (anatomical) cuttings were performed from freshly harvested young stems of *A. spinosus* L. (Amaranthaceae) and *B. erecta* (Nyctaginaceae).

For the identification of stem tissues, transverse cuttings were soaked in bleach for 15 min (to destroy the cellular content) and then rinsed three times. This was followed by a 5 min soak in 20% acetic acid to remove residual bleach. Following rinsing, the cuttings were soaked for 5 min in Mirande green Carmino colour and then rinsed with distilled water. Microscopic observations in glycerol were performed at a 40-fold magnification. Pectocellulosic cell walls were coloured pink, whereas lignin cell walls were coloured green. This analysis also allowed the observation of organic acid salt crystals.

For betacyanin detection, cuttings were treated with either hydrochloric acid or sodium hydroxide. In water, betacyanins are red, whereas in alkaline or acidic medium, they become yellow and blue, respectively.

Betacyanin extraction and quantification

The stem bark powder of the two species were extracted three times with 70% methanol (1/10; w/v), filtered, concentrated *in vacuo* and lyophilized. The betacyanins quantification was done by photometry, using the following absorption wavelength: 538 and 536, respectively for the extracts of *A. spinosus* and *B. erecta*.

Phenolic extraction and quantification

The stem bark powder was extracted with 70% acetone (1/10, w/v) filtered, concentrated and lyophilised. From aqueous solution of this extract was made a liquid-liquid partition with ethyl acetate previously adjusted to pH 1.5 with TFA to obtain the most lipophilic phenolic compounds of the extracts The total polyphenols and the more lipophilic phenolic were estimated by the method of Singleton (1999). This method assesses all the phenolic compounds reducing the phosphomolybdotungstic reagent (or Folin-Ciocalteu reagent, FCR). A volume of 1.25 ml of FCR (0.2 N in distilled water) was mixed with 0.25 ml of extract (0.1 mg/ml in distilled water). After 5 min, 1 ml of Na_2CO_3 (75 g/L) was added. The solutions obtained

were left to stand in the dark for 2 h. The absorbance was read at 760 nm and the values were used to determine the concentration using a calibration curve previously drawn with a series of dilutions of gallic acid (as a reference solution) solution. The results were expressed as mg gallic acid equivalents per 100 mg of extract (mg GAE/100 mg) using the following formula:

$C = c \times D \times 10/m$

where C = mg equivalent in 100 mg, c = reading concentration (mg/ml), D = dilution factor, m = mass of sample.

Measurement of antiradical activity

The activity of all extracts was measured by using the method of $ABTS^+$ (Thaiponga et al., 2006). The radicals were generated in a buffered reaction medium containing ABTS salt, horseradish peroxidase (HRP) (EC 1.11.1.7) and hydrogen peroxide. At the end point of the reaction, the extracts containing betacyanins or phenolics were added at different concentrations. After the addition of a test solution, the absorption (Ai) at 414 nm decreased until a constant absorbance (A_f) was reached asymptotically. The decay in absorbance (A_i-A_f) caused by the disappearance of $ABTS^+$ was used to estimate the antiradical activity of the tested compound. The percentage of lost radical activity was calculated by the following formula:

$$\text{Inhibition (\%)} = 1 - [A_{sample}/A_{blank}] \times 100$$

Asample is the radical absorbance with a sample solution. Ablank is the radical absorbance for the blank (buffer pH 7 for betalain, and methanol for phenolics). For each type of extract, the concentration value allowing reduction of 50% of the $ABTS^+$ radicals was determined, and has been called radical scavenging capacity of 50% (RSC_{50} µg/ml). For betalains and phenolics, the radical scavenging index (RSI), which is a composite measure of quantity and quality of antiradical activity, was determined. RSI was calculated by dividing the value of the total concentration of active compound, either betacyanin or phenolics (in mg/100 g of plant dried material) in the extract, by the RSC_{50} value (in µg/ml).

Statistical analysis

Statistical analyses were made using Microsoft Excel 97, and all results were expressed as the mean ± standard deviation (SD: n=3). The significance of the differences was calculated by a Student's t-test and values at $P<0.05$ were considered to be significant.

RESULTS

Histochemical location of metabolites

Analysis of the two plants' organs (leaves, stems and roots) showed that for both species, the stems contained most of the betacyanins. A comparison of Mirande green Carmino-coloured (after bleach treatment) and untreated cuttings (subjected to water, alkaline or acidic environments) showed that betacyanins were produced in the cortical parenchyma and could move to the epidermis depending on the physiological situation of the plant (Figure 1).

Detection tests with polyphenols reagents ($FeCl_3$ and $AlCl_3$) showed that these compounds are located more inner, in the parenchyma. The fact that betacyanins in the two species are located in the cellular vacuoles of sub-epidermis tissues can be connected to the ethnobotanical results showing the preferred medicinal use of the red specimens (Nacoulma-Ouedraogo, 1996). Indeed, plants generally accumulate their defensive secondary metabolites at the exposed organ periphery. That was also observed for alkaloids of many medicinal plants' stems or root barks (Richardson, 1981; Wink, 1999; Grassmann et al., 2002). Figure 2 compares green and red (betacyanin pigmented) stems of *A. spinosus* and B*. erecta*, respectively.

Phenolic and betalains levels

The results (Figure 5) obtained show that the stem bark of *B. erecta* is richer in phenolics and betacyanins than those of *A. spinosus*. The total phenolic content (2512 ± 28 and 3460 ± 42 GAE/100g of dried plant material, respectively for *A. spinosus* and *B. erecta*) and betalains (28.5 ± 1.21 and 193.9 ± 0.9 mg/100g of dried plant material, respectively for *A. spinosus* and *B. erecta*) show that these two species contain more phenolics than betalains.

Radical scavenging activity of different fractions of *A. spinosus* and *B. erecta*

For total phenolic fraction, the extract of *B. erecta* showed the highest radical scavenging activity (Figure 6). The RSC_{50} values were 171.2 ± 8.4 and 214 ± 10 µg/ml, respectively for *B. erecta* and *A. spinosus*.

The two species' phenolic extracts are less active than epigallocatechin (12. 2 µg/ml). Figure 2 shows that the betacyanin-containing fraction from *B. erecta* (5.54 ± 0.07 µg/ml) is more active than that of *A. spinosus* fraction (13.3 ± 0.11 µg/ml).

For both species, the betacyanins fractions are more active than those of phenolic compounds. The difference between the activities of *A. spinosus* betacyanins and those of *B. erecta* could also indicate differences in the structures of the betacyanin molecules (Figure 7).

DISCUSSION

These antiradical activity results show that betacyanins may play an essential role in the protection of these two plants against oxidative stress. The results can also be linked to the ethnobotanical (Nacoulma-Ouedraogo, 1996) data showing that reddish plants materials are

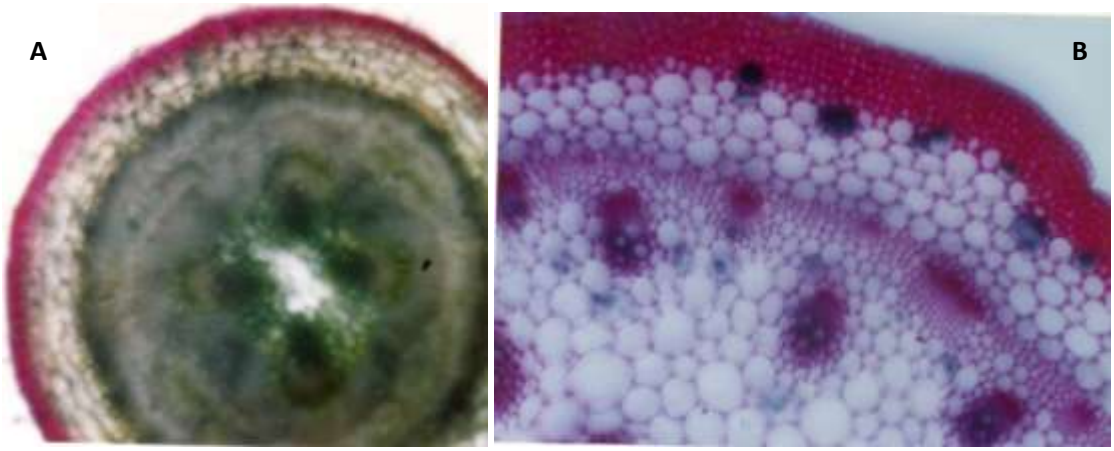

Figure 1. Chemohistological analysis on *B. erecta* (in water, image A) and *A. spinosus* (with green Mirande-Carmino colouring, image B) stems cuttings respectively.

Figure 2. Medicinal and non-medicinal plant material (stems) comparison; (A) for *A. spinosus:* medicinal (left) and non-medicinal (right) plant material; (B) for *B. erecta* : medicinal (left) and non-medicinal (right) plant material.

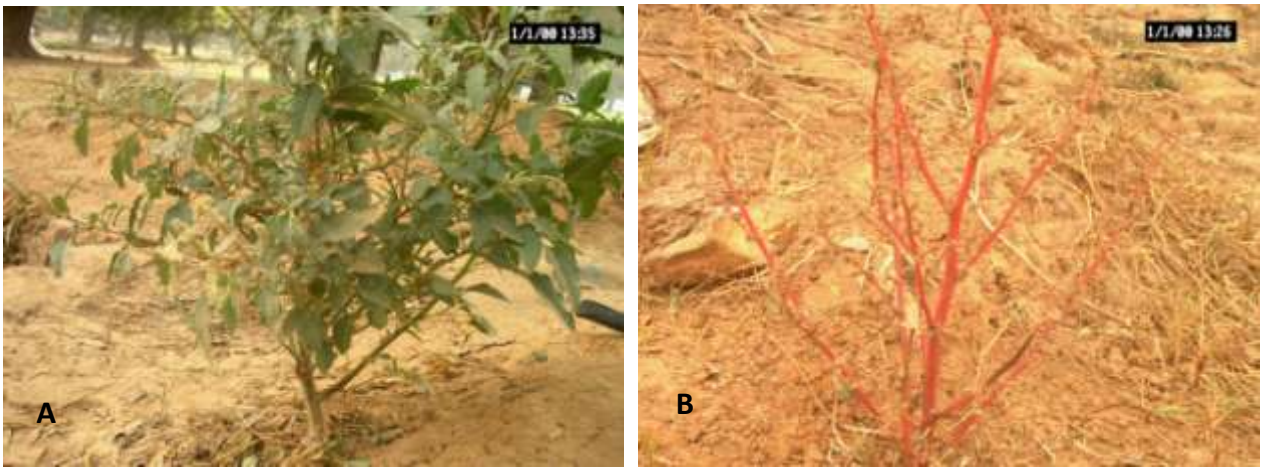

Figure 3. *A. spinosus* plants: (A) in august and (B) in december at Tanghin suburban of Ouagadougou, Burkina Faso.

Figure 4. *B. erecta* plants: (A) in august and (B) in december at Tanghin suburban of Ouagadougou, Burkina Faso.

preferred for ethnomedicinal uses. Field observations also showed that the pigmentation of both plants is maximal during biotic or abiotic-induced stress conditions (Figures 3 and 4).

Phenolic compounds and betalains are the main antiradical molecules of these two Caryophyllales species (Stintzing et al., 2004). The results of the present RSC_{50} data indicate that in the two species, these two classes of compounds have different level and antiradical activity.

Other studies (Butera et al., 2002; Stintzing et al., 2005) have also shown that this radical scavenging capacity of betalains, when assessed with the ABTS radical, could also indicate an antioxidant activity.

The results of Stintzing et al. (2004) showed that *A. spinosus* contains amaranthine, whereas *B. erecta* has betanin as betalain molecules. When a plant undergoes biotic or abiotic stress, a set of defence tools or responses is deployed. ROS such as hydrogen peroxide (H_2O_2), superoxide, hydroxyl radical and singlet oxygen, act as early messengers in signalling cascades activated by diverse external stimuli, such as pathogen attack, heat shock, mechanical stress or ultraviolet (UV) radiation (Schopfer et al., 2001; Sepulveda-Jimenez et al., 2004).

In plant-pathogen interactions, ROS may exacerbate tissue damage by a hypersensitive reaction in which programmed cell death (apoptosis) at the infection site restricts pathogen growth (Lamb and Dixon, 1997; Hansen, 2000). ROS can act as antimicrobial compounds and/or signalling molecules and can also induce cell wall strengthening (Borden and Higgins, 2002; Vranova et al., 2002). Subsequently, induction of genes encoding pathogenesis-related proteins occurs (Meier et al., 1993), as does ROS-detoxifying enzyme expression (Wink, 1999; Chong et al., 1999; Dat et al., 2000).

Among the ROS scavenging pathways, the production of antioxidants, such as ascorbic acid and glutathione, are essential to maintaining the physiological balance of ROS. Secondary metabolites (such as betacyanins in the case of the Caryophyllales species) are also necessary to either modulate ROS levels or to regenerate (by reduction) the oxidised small organic antioxidant molecules (e.g., ascorbate, glutathione) (Yamasaki et al., 1997; Cai et al., 2003).

Sepulveda-Jimenez et al. (2004) showed that mechanical wounding, microbial infection or exposure to a H_2O_2-generating system induced betacyanin biosynthesis in red beet leaves; they suggested that betalains work as ROS scavenger to aid in the plant's defence response.

In Caryophyllales plant species, the synthesis of betalains seems to be one of the best ways to protect DNA from stress. The results of histochemical study showed that betalains are located mainly in peripheral tissues (bark epidermis layer) of the parts most exposed to UV radiation. When exposed to sun, the roots of *A. spinosus* or *B. erecta* begin to pigment in red, after only a few days (results not shown). This fact can be connected to the work of Sarma and Sharma (1999) that showed that betalains like anthocyanins form copigmentation complexes with DNA molecules. All of these data show that betalains could be the most active compounds for the antioxidant activity of the extracts of *A. spinosus* and *B. erecta.* As for the mode of action of betalains, they could act as an antioxidant by one of the following mechanisms:

1) Reduction of organic (or not) free radicals by gift of hydrogen or electron (due to their phenolic groups); 2) Reduction or chelation of prooxidant metals (Attoe et al., 1984; Cheftel, 1977). This chelating activity would be amplified by their dicarboxylic pyridoxine functional group

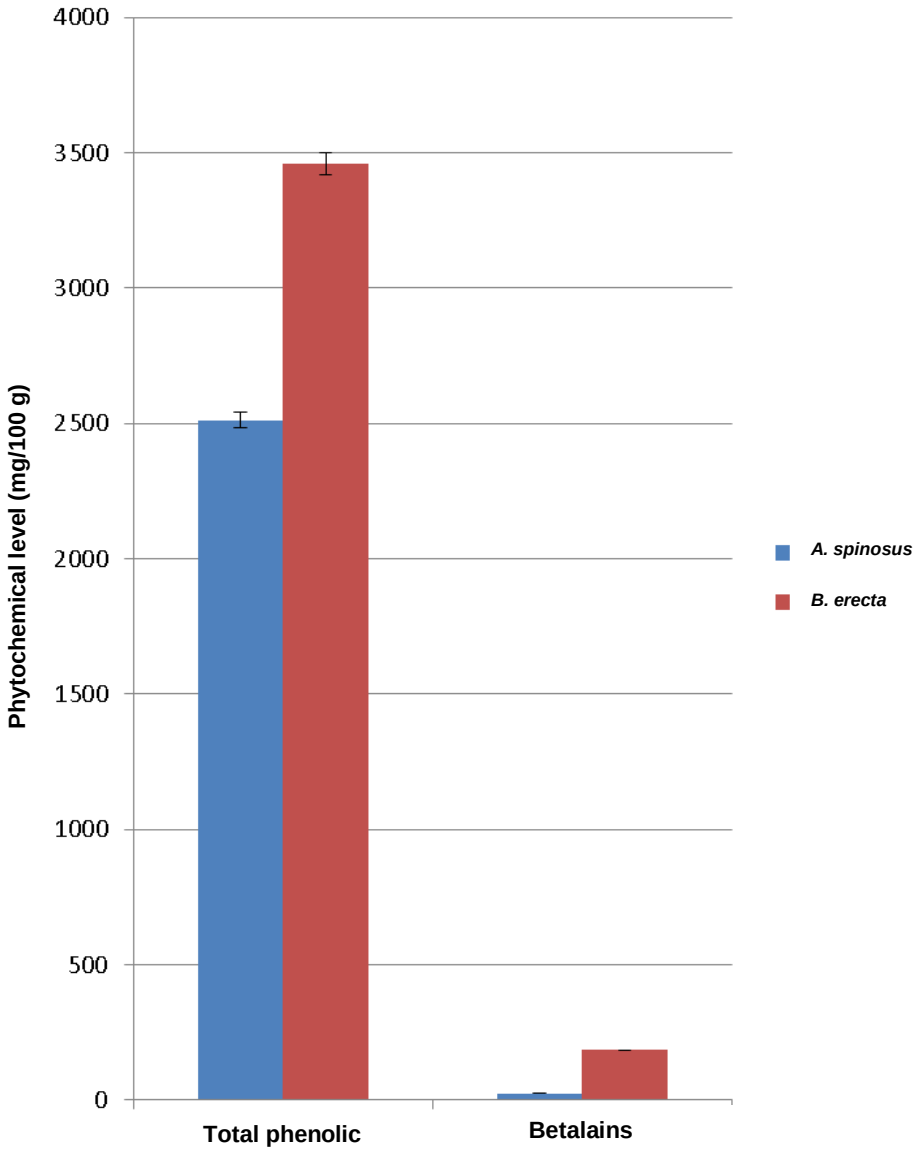

Figure 5. Phytochemical level of *A. spinosus* and *B. erecta* extracts.

(which is well known in ethylenediaminetetraacetic acid (EDTA)). Betalains also have a cyclic amine such as in ethoxyquin, a powerful antioxidant molecule used to stabilize lipids (Kanner et al., 2001).

The redox potential data (Butera et al., 2002) also allow to say that betalains reduce both lipopéroxyl and alkoxyls radicals. Voltammogram study also shows that oxidized forms of quinoline betalains cannot be reduced (by electron capture); in other words, they cannot become oxidants, because they are fairly stable (Martinez-Parra and Muñoz, 2001).

In comparison, studies show that flavonoids (best antioxidant phenolic molecules) act primarily as reducing agents and very little as chelators. Betalains (having phenol functional groups and cyclic amine) have two types of electron or hydrogen donating groups (Figure 3), while other phenolics have only one type. Moreover, cyclic amines are several times better active for free radicals reducing than phenols (Kanner et al., 2001).

Compared with vitamin antioxidants (also found in both species), the work of Tesoriere et al. (2005) showed that, against the atherogenic lipid peroxidation of low density lipoprotein (LDL) by myeloperoxidase (in the presence of nitrite), betanin is ten times more effective than vitamin C (1.4 and 15.6 µM as IC_{50} values, respectively for betanin and vitamin C).

During evolution, higher plants have developed self-defence mechanisms that protect them against environ-

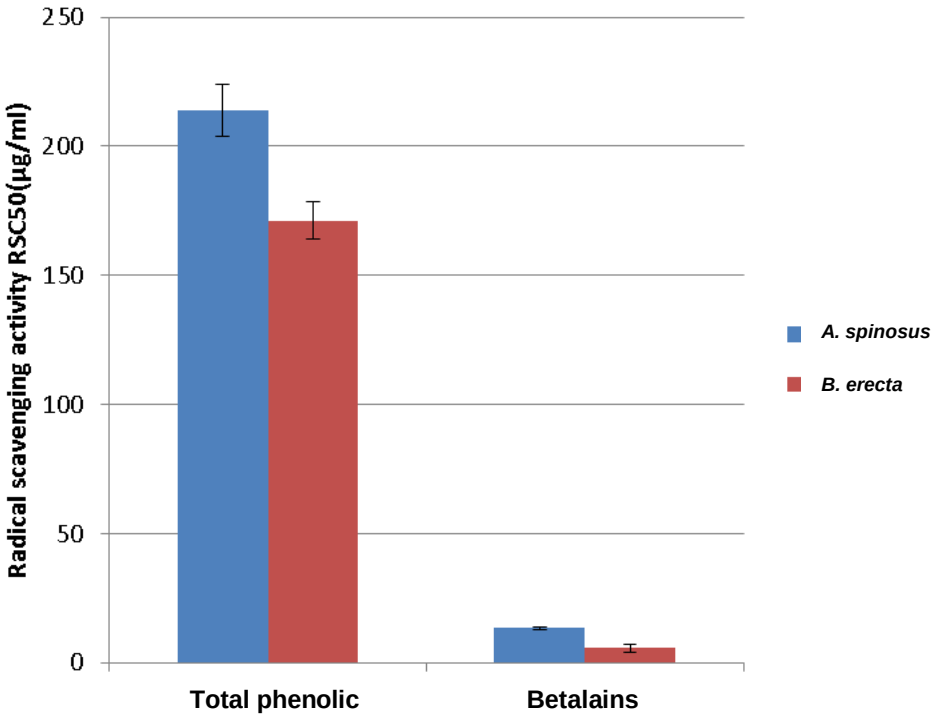

Figure 6. Antiradical activity of *A. spinosus* and *B. erecta* extracts

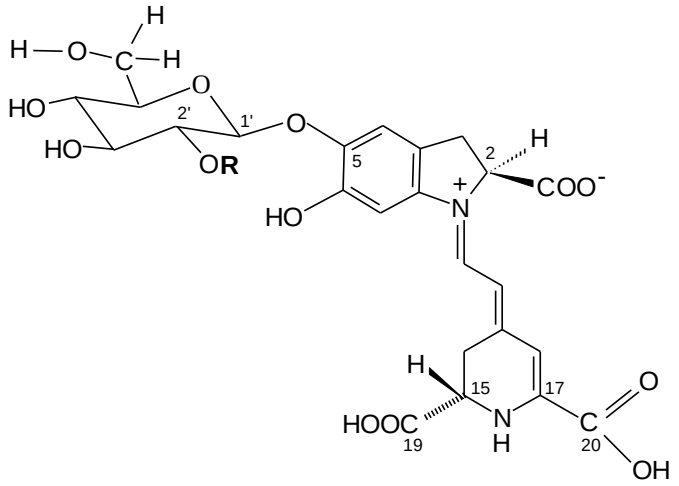

Figure 7. Chemical structure of two betacyanins from *A. spinosus* and *B. erecta*. R = Glucuronyl: Amaranthin; R = H: Betanin.

mental stress factors. Some of these mechanisms involve the synthesis of secondary metabolites, such as phenylpropanoids, terpenoids and alkaloids (Wink, 1999). The role of betalains in defence responses has been little studied thus far. Betalain biosynthesis by a plant non-reproductive organ can indicate that this plant (and the surrounding species) has been exposed to (and resisted) a stress (Kangatharalingam et al., 2002; Mittler, 2002); thus, it has activated its secondary metabolite synthesis mechanism (Richardson, 1981). Therefore, the specimen contains active metabolites for defence, although these substances might be different from betalains (Ditt et al., 2001).

Moreover, many studies have shown that betalains, like other metallic ion chelators (Elleingand, 1998; Kanner et al., 2001; Butera et al., 2002), have anti-parasitic activities. Thus, betalains are the more active antioxidant molecules of Caryophyllales species and their synthesis could serve as indicator of a plant good health.

Conclusion

The ethnobotanical, histochemical and biochemical activity revealed in this study show that betalains are the most active antioxidant compounds of *A. spinosus* and *B. erecta.* Betacyanin formation by a plant in stems or roots is most often indicative of its previous exposure to a stress (biotic or abiotic). If the plant survives this attack, it is obviously by defence secondary metabolites genes activation and encoding. Such plants have good potential to be used to prevent or treat human diseases. This fact may explain why the traditional healers of the Burkina Faso region of the “Plateau Central Mossi” use betacyanin presence in Caryophyllales species stems as a

biomarker for usefulness as folk medicine.

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REFERENCES

Attoe, EL, Von Elbe JH (1984). Oxygen involvement in betanin degradation: oxygen uptake and influence of metal ions. *Zeitschrift fur. Lebensmittel Untersuchung und forschung*, 179: 232-236.

Berghofer E, Schoenlechner R (2002). Grain amaranth. In: Belton, P.S., Taylor, J.R.N. (eds.), Pseudocereals and Less Common Cereals Grain Properties and Utilization Potential. Springer, Berlin, Heidelberg, New York, pp. 219–260.

Bloknina O, Virolainen E, Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a Review. Ann. Bot.-London, 91, 179-194.

Borden S, Higgins V (2002). Hydrogen peroxide plays a critical role in the defence response of tomato to *Cladosporium fulvum*. Physiol. and Mol. Plant Pathol. 61:227–236.

Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM (2002). Antioxidant activities of Sicilian prickly pear (*Opuntia ficus-indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agric. Food Chem.J; 50:6895–6901.

Cai Y, Sun M, Corke H (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. J. Agric. Food Chem. 51: 2288-2294.

Chalker-Scott L (1999). Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1–9.

Cheftel JC, Cheftel H, Besançon P (1977). *Introduction à la Biochimie et à la technologie des aliments*. Tome 2, Techniques et Documentation- Lavoisier, Paris.

Chong J, Baltz R, Fritig B, Saindrenan P (1999). An early salicylic acid-, pathogen- and elicitor-inducible tobacco glucosyltransferase: role in compartmentalization of phenolics and H_2O_2 metabolism. (Fed. Eur. Biochem. Soc. Lett.; 458:204–208.

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000). Dual action of the active oxygen species during plant stress responses. Cells Mol. Life Sci. 57:779-95.

Ditt RF, Nester EW, Comai L (2001). Plant gene expression response to *Agrobacterium tumefaciens*. Proc. Nat. Acad. Sci. USA 8:10954-10959.

Dogra JVV, Jha OP, Mishra A, Ghosh PK (1980). Chemotaxonomy of *Amaranthaceae*, Part II: Study of free amino and organic acids. Indian Bot. Soc. Proc. 59: 227–229.

Elleingand E, (1998). Réactivité du radical tyrosinyle de la ribonucléotide réductase: application à la recherche de nouveaux inhibiteurs. Thesis, University Joseph Fourier Grenoble I, France.

.Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine, 3rd ed.; Oxford University Press Inc.: New York.

Hansen G(2000). Evidence for *Agrobacterium*-induced apoptosis in maize cells. Molec. Plant-microbe Interact. 13:649–57.

Kangatharalingam N, Pierce ML, Bayles MB, Essenberg M (2002). Epidermal anthocyanin production as an indicator of bacterial blight resistance in cotton. Physiol. Mol. Plant Pathol. 61:189–95.

Kanner J, Harel S, Granit R (2001). Betalains: a new class of dietary cationized antioxidants. J. Agric. Food Chem. 2001; 49:51780–5185.

Lamb C, Dixon R (1997). The oxidative burst in plant disease resistance. Annu. Rev, Plant Physiol. Plant Mol. Biol. 48:251–275.

Mabry TJ (1980). Betalains. In: Bell EA, Charwood BV, editors. Encyclopedia of plant physiology. Secondary plant products, vol. 8. Berlin: Springer; p. 513–533.

Martinez-Parra J. Munoz R. (2001). Characterization of betacyanin oxidation catalyzed by a peroxidase from *Beta vulgaris* L. roots. J. Agric. Food Chem. 49(8): 4064-4068.

Meier BM, Shaw N, Slusarenko AJ (1993). Spatial and temporal accumulation of defense gene transcripts in bean (*Phaseolus vulgaris*) leaves in relation to bacteria-induced hypersensitive cell death. Mol. Plant-Microbe Interact. 6:453–66.

Mirales J., Noba K, Ba AT, Gaydou EM, Korn-probst JM (1998). Chemotaxonomy in Nyctagynaceae family: Sterols and fatty acids from the leaves of three Boerhaavia species. Biochem. Syst. Ecol. 16, 475-478.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science; 7:405–410.

Nacoulma-Ouedraogo OG (1996). Plantes médicinales et pratiques medicales traditionnelles au Burkina Faso: cas du Plateau central Tomes I & 2. Thèse de Doctorat d'Etat ès sciences Naturelles. Université de Ouagadougou.

Richardson PM (1981). Phytoalexin induction in *Beta* and *Spinacia*. Biochem.Syst. Ecol.; 9:105–7.

Samy RP, Ignacimuthus S, Raja DP (1999). Preliminary screening of ethnomedicinal plants from India. J. Ethnopharmacol, 66: 235-240.

Sarma A D , Sharma R (1999). Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. Phytochemistry, 52: 1313-1318.

Schopfer P, Plachy C, Frahry G (2001). Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidases in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol.; 125: 1591–602.

Sepulveda-Jimenez G, Rueda-Benıtez P, Porta H, Rocha-Sosa M (2004). Betacyanin synthesis in red beet (*Beta vulgaris*) leaves induced by wounding and bacterial infiltration is preceded by an oxidative burst. Physiol. Mol. Plant Pathol. 64: 125–133.

Singleton V L, Orthofer R, Lamuela-Raventos RM (1999). Analysis of phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalceu Reagent. Methods in Enzymol. 299: 152-178.

Srivastava R, Shukla YN , Kumar S (1998). Chemistry, pharmacology and botany of *Boerhaavia diffusa*- a review. J. Med. Aromatic Plants Sci. 20: 762-767.

Stafford HA, (1994). Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Sci.; 101:91–98.

Stintzing F C, Kammerer D, Schieber A, Hilou A, Nacoulma O, Carle R (2004). Betacyanins and phenolic Compounds from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. Zeitschrifts fur Naturforsch., 59c: 1-8.

Stintzing FC, Herbach KM, Mosshammer MR, Carle R, Yi W, Sellapan S, Ako HCC, Bunch R, Felker P (2005). Color, Betalain pattern and antioxidant properties of cactus pear (*Opuntia Spp*.) clone. J Agric. Food Chem., 2: 442-451

Strack D, Vogt T, Schliemann W, (2003). Recent advances in betalains research. Phytochemistry; 62:247–269.

Tesoriere L, Butera D, Arpa D, Di Gaudio F, Allegra M , Gentile C. (2005). Increased resistance to oxidation of betalain-enriched human low-density lipoproteins. Free Radical Research, 37(6): 689-696.

Thaiponga K, Boonprakoba U, Crosbyb K, Cisneros-Zevallosc L, Byrnec DH (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19

Vogt T, Ibdah M, Schmidt J, Wray V, Nimtz M, Strack D(1999). Light inducedbetacyanin and flavonols accumulation in bladder cells of *Mesembryanthemum crystallinum*. Phytochemistry; 52:583–592.

Vranova E, Inze D, Breusegem FV (2002). Signal transduction during oxidative stress. J. Exp. Bot.; 53:1227–36.

Wink M (1999). Introduction: biochemistry, role and biotechnology of secondary metabolites. In: Wink M, ed. Biochemistry of plant secondary metabolism. England: Sheffield Academic Press; P.1–16

Yamasaki H, Sakihama Y, Ikehara N, (1997). Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. Plant Physiol.; 115:1405–12.

Zhao BL (2005). Natural antioxidants for neurodegenerative diseases. Mol. Neurobiol. 31, 283- 293