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Research Article

PHENOLIC COMPOUND OF BENIN'S RED *SORGHUM* AND THEIR ANTIOXIDANT PROPERTIES

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ABSTRACT

The antioxidant activities and phenolic content of various organs (grain, stalk and foliar sheath) of the red *sorghum*, a tinctorial and medicinal plant of Benin, were determined. The total phenolic content, determined according to the Folin-Ciocalteu method, varied from 19.8 to 233.5 mg/g of dry product whereas anthocyanin content expressed as mg of malvidin equivalents per g of sample (dry weight basis) lies between 28 and 115% of anthocyanin (monomeric form) contained in the grain against 70% and 50%, respectively, for the foliar sheath and the stalk.

Antioxidant activity of aqueous ethanolic extracts compared with aqueous extracts, evaluated according to the DPPH method, showed a higher antioxidant activity of aqueous ethanolic extracts. Moreover, all of those samples also contained higher levels of phenolic compounds. Thus, the correlation between total phenolics and antioxidative activities of extracts has been noted. Also, the presence of organic solvent favored the extraction of more bioactive compounds from vegetables materials.

Keywords: antioxidant activity, DPPH, *Sorghum caudatum*, foliar sheath, stalk, grain...

INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^-), peroxyl radical (ROO.) and nitric oxide radical (NO.), attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury. ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidants like heptanol and hexanal which contributes to oxidative rancidity, deteriorating the flavor of the food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity. To overcome these problems a wide range of synthetic antioxidants (butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG) and butylatedhydroquinone) have been used as food preservatives. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin [1]. Recently, there have been increasing interests in discovery of natural antioxidants, especially those of plant origin. Natural antioxidants derived from plants, chiefly phenolics are of considerable interest as dietary supplements or food preservatives. Flavonoid is one of the main groups of phenolic compounds and many of them are reported to possess strong antioxidative characteristics [2]. In the present study, we have selected the plant *Alocasia decipiens* Schott, which is a member of family Araceae, to assess the antioxidant potential of its rhizome. We have also attempt to determine the phenolic and flavonoid content of the rhizome, as these are reported to be natural antioxidants present in plant

MATERIALS AND METHODS

Plant Material

The stalk, foliar sheath and grain of red cultivars of *Sorghum caudatum* (L.) Moench (Poaceae) were harvested in Bassila (Benin) in June 2009. Samples were dried in shade and keep from light until mass stabilization.

Reference Compounds

Gallic acid, butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and (-)-catechin were purchased from Sigma Chemical Co. (St. Louis, MO) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Acros Organics (Morris Plains, NJ). All solvents were reagent grad

Sample Extraction.

All samples were ground through a commercial coffee grinder to extraction. Two extraction solvents were used: distilled water and the mixture ethanol-water 50% (v/v). The extraction procedure involved the addition of 50 mL of solvent to 1 g of sample in 100 mL centrifuge tubes and shaking the samples for 2 h at low speed in a shaker. Samples were then centrifuged at 7000 *rpm* for 10 min and decanted. Residues were rinsed with two additional 25 mL volumes of solvent with shaking for 5 min, centrifuging at 7000 *rpm* for 10 min, and decanting in each case. The three aliquots were combined before storage at -20 °C in the dark.

Total phenolic compounds and tannins content

Samples were analyzed for phenolic content using the modified Folin-Ciocalteu method of Kaluza et *al.* [16]. The tannin contents were determined using the modified Vanillin-HCl method [17] with and without blank subtraction. Catechin was used as a standard.

Total anthocyanin and monomeric anthocyanin content

The pH differential method reported by Fuleki *et al.* [18] and by Giusti and Wrolstad [19] was used for quantitative determination of anthocyanin with minor modifications. One of two 0.2 mL aliquots was diluted with 2.8 mL of pH 1.0 buffer (125 mL of 0.2N KCl, and 385 mL of 0.2 N HCl) and the other with pH 4.5 buffer (400 mL of 1N sodium acetate, 240 mL of 1N HCl, and 360 mL of distilled water). The absorbance was measured by scanning with a Cary 300 Bio UV-Vis spectrophotometer (Varian Co., Walnut Creek, CA) from 200 to 800 nm. Total anthocyanin pigments were determined from absorbance in pH 1.0 buffer. Extinction coefficients for anthocyanin standards were determined using the formula described by Fuleki, et *al.* [18]. All analyses were replicated three times. The monomeric anthocyanin pigments content in samples was expressed as apigenidin equivalent, according to the formula published in the literature [20].

pH Stability of anthocyanins

In order to detect the presence of anthocyanins in crude extracts, a simple test of variation of pH of extracts as reported by Eloi Pale and Mouhoussine Nacro[21] was applied. Two test tubes (T_1 and T_2) were chosen and 2 ml of a 1N HCL solution was put in T_1 and 2 ml of a 1N NaOH solution in T_2. Few drops of the concentrated solution of crude extract were added in T_1 and T_2 and the color changes were noted.

UV-Visible spectrometry analysis

Absorbance was measured from 200 to 800 nm in a Cary 300 Bio UV-Vis spectrophotometer (Varian Co., Walnut Creek, CA) according to method previously described by Eloi Palé and Mouhoussine Nacro [21].

Antioxidant activity

The antioxidant activity of the aqueous ethanolic extracts, aqueous extracts, BHA, BHT and gallic acid was assessed by measuring free-radical scavenging activity *via* the decoloration of a methanol solution of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Williams et *al.*[22] as follows : 2 mL of methanol solution of each tested material at various concentrations (2-50 µg/mL) were added to a 2 mL solution of DPPH (100 µM) in methanol, and the reaction mixture was shaken vigorously. After incubation at room temperature for 30 min, the absorbance (A) of DPPH was determined with a spectrophotometer at 515 nm, and the radical scavenging activity of each sample was expressed as percentage inhibition:

% inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100$

IC50 (sample concentration required for 50% inhibition) was obtained by linear regression analysis of the dose response curve, plotted as the % inhibition and concentration (mg/L). Gallic acid, which is the better well-known natural antioxidant, was used as a positive control.

Results and discussion

pH stability of anthocyanins

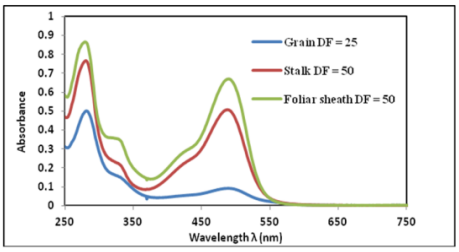
The samples of grain and stalk appear respectively in yellow and orange color at acidic pH (T_1) and turn into red in basic medium (T_2). According to the literature [21,23,24], these observations indicate the presence of 3-deoxyanthocyanidins in those parts of the plant. Also, the solution of foliar sheath in T_1 was yellow and dark red in the T_2. A precipitate was also observed in T_2. Thus, we can conclude that the foliar sheath has a high concentration in 3-deoxyanthocyanins.

Table1: Change in color of crude extracts at variable pH

	color in T_1(acidicaqueous medium)	T_2 (basic aqueous medium)
grain	yellow	red
stalk	orange	red
foliar sheath	yellow	dark red

UV-Visible spectrometry analysis

According to Eloi Pale and Mouhoussine Nacro [21], UV-Visible spectrometry is useful for getting important information in the identification of anthocyanin pigments. The spectra recorded for three extracts, show two characteristic absorption bands located around 280 nm, due to the phenolic group, and around 470-560 nm due to the pyrylium nucleus and the conjugation of the two benzene rings. The maximum of absorption in the visible located around 490-498 nm should be related to 3-deoxyanthocyanins. The absorption band between 300-335 nm indicated that anthocyanins contain an aromatic acid residue.



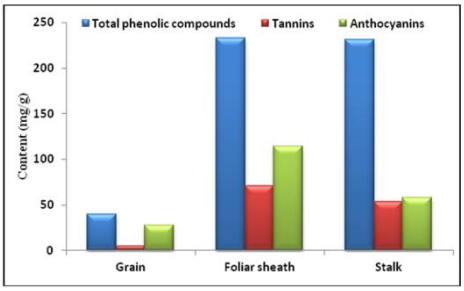
DF = Dilution factor

Figure 1: UV-Vis spectra of three extracts

As mentioned in Table 1, all organs of the plant (grain, stalk and foliar sheath) contain 3-deoxyanthocyanins. This plant could therefore constitute a good source of anthocyanin and especially 3-deoxyanthocyanin which has a small distribution in nature [25], and is distinct from the more widely distributed anthocyanidins due to the absence of oxygen at the C-3 position. These 3-deoxyanthocyanidins were reportedly as a very stable in acidic solutions relative to the anthocyanidins, commonly found in fruits and vegetables [26]. The absence of oxygen at C-3 might improve their stability. This property points to the potential advantage of sorghum over fruits and vegetables as a viable commercial source of anthocyanins. According to literature, the most common anthocyanins in sorghum are 3-deoxyanthocyanidins [26,27], as luteolinidin and apigeninidin [28].

Total polyphenol, tannins and anthocyanins content

Figure 2 shows that stalk (233.5 mg/g of GAE) and foliar sheath (231.4 mg/g GAE) give similar high concentration in phenolic compounds compared to grain. As regards to the presence of anthocyanin and tannins in three organs, it is clear that anthocyanin and tannins are located in foliar sheath and stalk; however, grain contains much lower concentrations of these substances.



Total phenolic compounds expressed as mg of Gallic acid equivalents per g of sample (dry weight basis).
Tannins expressed as mg of catechin equivalents per g of sample (dry weight basis).
Anthocyanins expressed as mg of malvidin equivalents per g of sample (dry weight basis).

Figure 2: Total polyphenols, tannins and anthocyanins contents of three extracts

Monomeric anthocyanin content

As can be seen from Table 2, foliar sheath contains twice of monomeric anthocyanins in comparison with the stalk that contains ten times more than the grain.

These results compared with total polyphenol content showed that sorghum grain contains more phenolic compounds, except from anthocyanins, present in the other plant organs in two forms : monomeric and proanthocyanidins. Over 85% of anthocyanins from the seed are in monomeric form, whereas in the foliar sheath and the stalk, 70% and 50% are respectively in monomeric form.

Consequently, proanthocyanidin content is more important in foliar sheath and stalk than in seed. Extraction of monomeric anthocyanins with aqueous methanol ($MeOH/H_2O$ 1/1) is far more efficient than extraction with water: twice as much from the foliar sheath, and respectively, four times and six times more from the stalk and the grain.

Table 2: Monomeric anthocyanin content (mg of apigenidin equivalent/g of dry mater)

	aqueous extracts	$EtOH/H_2O$(1/1) extracts
grain	0.3	2.0
stalk	3.5	14.3
foliar sheath	7.6	17.4

Antioxidant activity

Stability and linear range of DPPH solutions were evaluated by UV-Visible analysis of five methanol solution of DPPH (from 0.015 to 0.25 mg/ml).

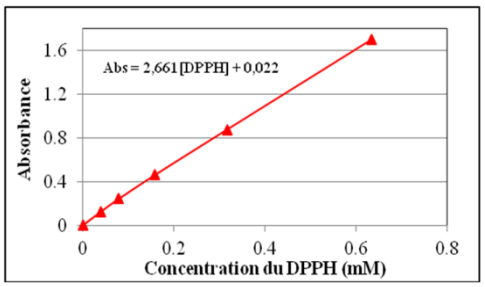


Figure 3: Calibration curve of DPPH

Whatever the DPPH concentration considered, there is no significant difference in absorbance between 0 and 90 min for the tested concentrations and a good linearity concentration-absorbance was observed. According to Ozcelik [29], the absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 517 nm in methanol decreases by 20% after 120 min at room temperature, whereas no significant change was reported after 150 min in the dark. We confirmed this result with the calibration curve of DPPH (Figure 3) reflecting the evolution of the absorbance versus concentration which is almost confused with the trend curve

All the extracts were significantly active as antioxidant agents. The results of DPPH tests for extracts of the three organs of sorghum and those of the standards are listed in Table 3.

Gallic acid was chosen as one of the most active standard to compare the results of the sorghum samples.

The aqueous ethanolic extract of three samples exhibited better free radical scavenging action against 1,1-diphenyl-2-picrylhydrazyl (DPPH). Aqueous ethanolic extracts of three bodies were more efficient as an antioxidant than gallic acid since their IC_{50} values were lower than those of gallic acid (Table 3). IC_{50} was defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. According to literature lower is the IC50 value better is the DPPH radical scavenging activity

Foliar sheath and stalk show similar high activities compared to the grain. In this study we, pointed out the relationship between total polyphenol content and antioxidant activity of sorghum caudatum extracts (already mentioned in literature in the cases of *Polysiphonia urceolata and Gracilaria changii)*. These results are similar to that of Duan *et al*. [30] who found a significant correlation between the total phenolic content and the antioxidant activity in extract of *Polysiphonia urceolata*. Same results was obtained by Sreenivasan et *al*. [31] and Faten et *al.*[32] who evaluated respectively the antioxidant activity and phenolic content of *Gracilaria changii* on the one hand and Antioxidant Activity of Extract and Semi- Purified Fractions of Marine Red Macroalga, *Gracilaria Verrucosa* on the other hand.

Table 2: Activities (IC_{50} expressed in g/mol of DPPH) of aqueous and aqueous alcoholic extracts of three organs of sorghum

	grain	stalk	foliar sheath	BHA	BHT	gallic acid
aqueousethanolic extract	4	3.1	3	19.9	99.1	**8**
aqueous extract	120	22	62			

CONCLUSION

In this study, we reported *sorghum* foliar sheath and stalk as potential sources of antioxidant components. Antioxidants are well-known to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation induced by oxidative stress or for use as food additive to delay the oxidative deterioration of foods.

The aqueous ethanolic extracts of *sorghum caudatum* have shown higher activity than aqueous extracts and this strong antioxidant activity could be related to the activity of phenolic compounds. Further studies are needed to investigate *in vivo* pharmacological and toxicological properties of *sorghum caudatum* extracts, since the high activity could be considered as a new antioxidant ingredient for the nutraceutical or functional food market.

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