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ORIGINAL RESEARCH PAPER

PHYTOCHEMICAL STUDY OF A TINCTORIAL PLANT OF BENIN TRADITIONAL PHARMACOPOEIA: THE RED SORGHUM (*Sorghum caudatum*) OF BENIN

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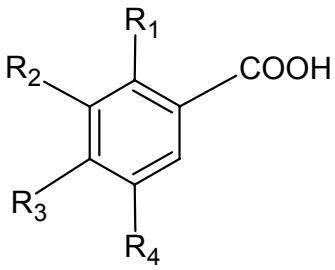
Abstract: The full phytochemical screening of red sorghum from Benin (*Sorghum caudatum*) achieved in this work reveals the presence of leucoanthocyanins, flavonoides, free quinones, combined anthracene derivatives, sterols and terpenes in higher concentration in the leaf sheath and marrow of stem than in the seed. Catechin tannin content is 11.4% in the leaf sheath (slightly higher than that of red wine), 5.8% in the marrow and 2.8% in the seed. Gallic tannins, saponins and the mucilage present in the leaf sheath and marrow, are virtually absent in the seed. Marrow and leaf sheath extracts (1 g/50 mL) showed a concentration of anthocyanins (147 mg/L and 213.5 mg/L) similar to that of rosy wine and red wine with short maceration. The grain of sorghum is four times, respectively two times less rich in phenolic compounds than the leaf sheath and the marrow of stem.

Keywords: *agroalimentary, anthocyanins, foliar sheath, grain, marrow of stem, phenolic compounds, Sorghum caudatum*

INTRODUCTION

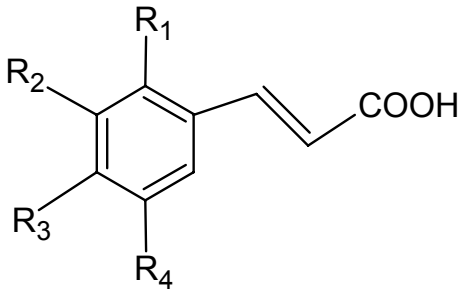
The importance of the sorghum is well known, either as poultry and cattle feed, either for human consumption, being an amidonous plant free of gluten. World production of sorghum is about 57 million tons and ranks fifth, after that of maize, rice, wheat and barley [1]. Sorghum (*Sorghum caudatum* L.) is one of the major cereal food crops in many parts of the world and is particularly important as a human food resource and folk medicine in Asia and Africa [2-4]. According to FAO report in 1996 on the Benin phytogenetic resources, sorghum occupies the fourth row among the principal cultures of Benin after yam (700,000 - 4,000,000 tons), manioc (600,000 - 1,000,000 tons) and maize (35,000 - 105,000 tons) and comes in the second position among cereals [5].
More studies worldwide have shown its importance in human health. Among the biologically active molecules isolated from extracts of sorghum include:

- phenolic acids: hydroxybenzoic (Figure 1) and hydroxycinnamic (Figure 2) acids (Table 1);
- flavonoids: anthocyanins, flavan-4-ol, flavones, flavanones, flavonols, proanthocyanidins and tannins (Table 2);
- phytosterols and policosanols [3, 6];
- stilbenes: trans-resveratol and trans-piceide [6-8].



hydroxybenzoic acids	R_1	R_2	R_3	R_4
gallic acid	H	OH	OH	OH
gentisic acid	OH	H	H	OH
salicylic acid	OH	H	H	H
p-hydroxybenzoic acid	H	H	H	OH
syringic acid	H	OCH_3	OH	OCH_3
protocatechic acid	H	OH	OH	H

Figure 1. *Structure of hydroxybenzoic acids*



hydroxycinnamic acids	R_1	R_2	R_3	R_4
cafeic acid	H	OH	OH	H
ferulic acid	H	OCH_3	OH	H
o-coumaric acid	OH	H	H	H
p-coumaric acid	H	H	OH	H
sinapic acid	H	OCH_3	OH	OCH_3

Figure 2. *Structure of hydroxycinnamic acids*

Several biological activities and pharmaceutical benefits of these phytochemicals in sorghum have been reported [27, 28]. Recent studies have shown that sorghum has antioxidant activity, anticarcinogenic effects, cholesterol-lowering effects, antioxidant and antimicrobial activities and can reduce the risk of cardiovascular disease and the LDL oxidation [1, 2, 29-32].
Nevertheless, almost all those works concerned the seed of sorghum; little information is available concerning the foliar sheath of sorghum. In Benin, except a study related to genetic and environmental impact on the composition of iron, zinc and phytate in various Benin red sorghum [33], the plant is badly known on a scientific level.

Table 1. *Phenolic acids identified in sorghum*

Phenolic acids	References
Hydroxybenzoic acids	
gallic acid	9, 10
protocatechic acid	9 - 11
p-hydroxybenzoic acid	9, 11
gentisic acid	11, 12
salycilic acid	12
vanillic acid	9 - 11
syringic acid	11, 12
Hydroxycinnamic acids	
ferrulic acid	9 - 11
cafeic acid	9 - 11
p-coumaric acid	9 - 11
cinnamic acid	9, 11
sinapic acid	11, 12

Table 2. *Flavonoids and proanthocyanidins found in sorghum*

Flavonoids	Ref.	Flavonoids	Ref.
Anthocyanins		*Flavanones*	
apigenidin	13 - 15	eriodictyol	21
apigenidin-5-glucosid	13, 16, 17	eriodictyol-5-glucosid	24
luteolidin	13 - 15	naringenin	24, 31
5-metoxyluteolidin	17, 18	*Flavonols*	
5-metoxyluteolidin 7-glucosid	17	kaempferol 3-rutinosid 7-glucuronid	13
7-methoxyapigenidin	17, 19	*Dihydroflavonols*	
7-methoxyapigenidin 5-glucosid	17	taxifolin	24
luteolidin 5-glucosid	3, 17	taxifolin 7-glucosid	24
5-methoxyapigenidin	18	*Monomeric/dimeric proanthocyanidins*	
7-metoxyluteolidin	18	catechin	24, 25
Flavan-4-ol		procyanidin B-1	24, 25
luteoforol	20 - 22	*Polymeric proanthocyanidins*	
apiforol	22, 23	epicatechin-(epicatechin)n-catechin	24, 25
Flavones		prodelphinidin	26, 27
apigenin	18, 24	proapigenidin	27
luteolin	18	proluteolidin	27

However, the extracts of its foliar sheath constitutes a very effective remedy against anemia. Thus we conducted this phytochemical study of Benin red sorghum in order to better know its principal components and to better understand the pharmacodynamic properties of its extracts for potential applications.

MATERIALS AND METHODS

Herbal materials

Various parts (foliar sheath, seed and marrow of stem) of *Sorghum caudatum* were harvests in the village of Kpakpassa located 12 km away from the town of Savalou in the center of Benin. A specimen was deposited in the National Herbarium of the

Department of Botany, Abomey-Calavi University. Samples were dried in shade and keep from light until stabilization of their mass.

Chemical reagents and standards

Ethanol (97%) and formic acid (p.a.) were obtained from Aldrich (Germany). Acetonitrile (99.99%), toluene (97%) and cyclohexane (99.96%) were supplied by Fisher Scientific (U.K.). Methanol (99.9%) and diethyl ether (99%) were supplied by Romil (Cambridge, U.K.). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA) double-distilled water (resistance = 18 MΩ/cm^2). (–) Epicatechin, (+) catechin, epigallocatechin and taxifolin were purchased from Sigma Chemical Co. (St Louis, MO). Chromatographic plates (standard silica gel Camag CH-4132 Mutteng, plate of 200 × 200 mm, 0.3 mm thickness, plastic support) and all other reagents used are analytical quality.

Phytochemical screening

We explored the potential chemicals of the various parts of the plant by a series of coloring reactions, precipitation reactions and thin layer chromatography.

Alkaloids

Three various properties based on the capacity of alkaloids to combine with heavy metals or iodine (Dragendoff's reagent, Mayer's reagent and iodoplatinate test) were implemented [34].

Coumarins

The characterization of coumarins was made according to the method described by Rizk [35].

Saponosides or saponins - foaming index

Two grams of dry and ground *Sorghum caudatum* were used to prepare a decoction with 100 mL of distilled water and submitted to boiling for 30 min, then the resulting solution was divided in 10 tubes (1.3 cm in diameter inside): 1, 2, 3, …, 10 mL of decoction. The content of each tube was adjusted to 10 mL with distilled water. Each tube was shaken vigorously in a horizontal position for 15 seconds. After 15 min in vertical position, persistent foam measurement was performed. If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100. If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. The foaming index was calculated by the following formula:

$$FI = \frac{1000}{a}$$

where a is the volume (mL) of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed. The presence of saponins was confirmed by an index exceeding 100 [36].

A qualitative approach on methanolic extracts, prepared according to the method described for research of alkaloids, was done by TLC with AcOEt : MeOH : H_2O (100 : 13.5 : 4) as solvent of migration, and was visualized with sulfuric vanillin.

Sterols and terpenes

Sterol and terpenes were identified by Liebermann-Buchard reaction [36].

Carotenoids and quinones

We carried out the characterization of carotenoids by the reaction of Carr and Price, free anthraquinones by the reaction of Bornträger [37], combined quinones (O-heteroside and C-heteroside) by methods of characterization usually used in our laboratory.

Polyphenols

The phenolic compounds such as tannins (gallic and catechic tannins), and flavonoids (anthocyanins, free genines, leucoanthocyanins) and the mucilages were characterized by traditional methods available in our laboratory [34, 38, 39].

Quantitative analysis of polyphenols

Anthocyanidins or anthocyanidols

After extraction and purification according to Lebreton method [40], anthocyanidins were subjected to two types of analysis:

The qualitative analysis was carried out by thin layer chromatography (TLC) on paper Wathman N°1 in forestal as solvent of migration and was visualized under visible light.

For quantitative analysis, the absorbance of extracts was measured at the maximum wavelength (λ_{max}) using the spectrophotometer and anthocyanidin content was calculated by following formula described in literature [40]:

$$\text{Anthocyanidins}_{Total} = \frac{\gamma \cdot A \cdot M \cdot V \cdot f}{\varepsilon \cdot P} \quad \text{(mg of anthocyanidin/g of dry matter)}$$

where: γ - factor of correction (γ = 6) of proanthocyanidins transformation output (17%); A - absorbance at the maximum wavelength; ε - molar absorption coefficient of the cyanidol (34700 $L \cdot mol^{-1} \cdot cm^{-1}$); M - molar mass of leucocyanidol (306 g/mol); V - volume of butanolic solution (mL); f - dilution factor; P - mass of dry vegetable material (g).

Flavonic aglycones

According to the same method described above for anthocyanidins analysis, the flavonic aglycones were extracted and purified.

The qualitative analysis was carried out by thin layer chromatography (TLC) on silica gel and was visualized under UV (254 nm), using $CHCl_3$: MeOH : H_2O : AcOH (100 : 15 : 0.5 : 0.3) as eluting solvent.

The differential proportioning of flavones and flavonols was carried out based on their chelating properties with $AlCl_3$ 1% in ethanol 95%. The absorbance was measured by using a UV–Vis spectrophotometer (Varian-Cary 5000, equipped with a double beam) at the wavelength of 380 nm to 460 nm. The differential height of the peaks against a sample consisting of an extract solution with ethanol 95% without $AlCl_3$ is proportional to concentration of flavonic aglycones in the sample. The flavones have a maximum of

absorption between 390 and 415 nm, whereas that of flavonols lies between 420 and 440 nm [41].

$$\text{Aglycones}_{Total} = \frac{A \cdot M \cdot V \cdot f}{\varepsilon \cdot P} \text{ (mg of aglycones/g of dry matter)}$$

where: A - absorbance of differential peaks; ε - molar absorption coefficient of the quercetol (23000 $L \cdot mol^{-1} \cdot cm^{-1}$); M - molar mass of quercetol (302 g/mol); V - volume of ethanolic solution (mL); f - dilution factor; P - mass of dry vegetable material (g).

Anthocyanins

The quantitative analysis of anthocyanins was carried out using two different properties based on their structures:

- The change of their colors depending on the pH. 3-oxyanthocyanidins and their derivatives show a red color in acidic medium and a blue color in basic solution, while 3-deoxyanthocyanidins and their derivatives are yellow or oranges in acidic medium and red in basic solution [38].
- The transformation into colorless derivatives under the action of certain reagents like bisulfite ions. Thus, variation of the absorbance at 520 nm after addition of excess bisulfite ions is proportional to the anthocyanins.

The total content of anthocyanins expressed as mg/L of standard of extract is given according to the formula [42]:

$$\text{Anthocyanins}_{Total} = (OD_A - OD_B) \times P$$

where: OD_A - absorbance of witnesses solution (without bisulfite); OD_B - absorbance of the mixture; P - line slope obtained starting from the standard; (P = 875 for malvidine-3-glucoside).

RESULTS

Phytochemical screening

The result of phytochemical screening of three parts (foliar sheath, seed and marrow of stem) of Benin red sorghum (*Sorghum caudatum*) is summarized in Table 3.

Polyphenols analysis

Anthocyanidins

The anthocyanidins content of the three bodies of sorghum was proportioned in accordance with the protocol describes in the experimental part. The graph in Figure 3 translates the obtained results.

The qualitative analysis (Table 4) showed a small yellow spot in the seed extract, two spots in the marrow of stem extract of which one of strong and the other of low intensity, a spot of strong abundance and one of average abundance in the foliar sheath extract. UV-Vis spectra of rough extracts are presented in Figure 4.

Table 3. *Secondary metabolites of different parts of* ***Sorghum caudatum***

Components		Foliar sheath	Seed	Marrow of stem
Tannins	total tannins content ($FeCl_3$ reaction)	+ + +	+ +	+ + +
	cathechic tannins	+ + +	+ +	+ + +
	gallic tannins (Stiasny reaction)	+ +	–	+ +
Flavonoids	anthocyanins	+++	+	++
	free flavonoids (cyanidin reaction)	+++	+++	+++
	leucoanthocyanins	+++	+++	+++
Saponins	foam height	+++	traces	+++
	foam index	500	‹ 100	250
Sterols and terpenes		+++	++	++
Alkaloids		++	–	traces
Coumarines		–	–	–
Mucilages		+++	traces	traces
Free quinones		++	+	++
Combined anthraqui-nones	O-heteroside	+++	+	+++
	O-heteroside with reduced genine	–	+	–
	C-heteroside	+	++	+

+ : relative intensity;
: negative result; : positive or negative result; : positive result.

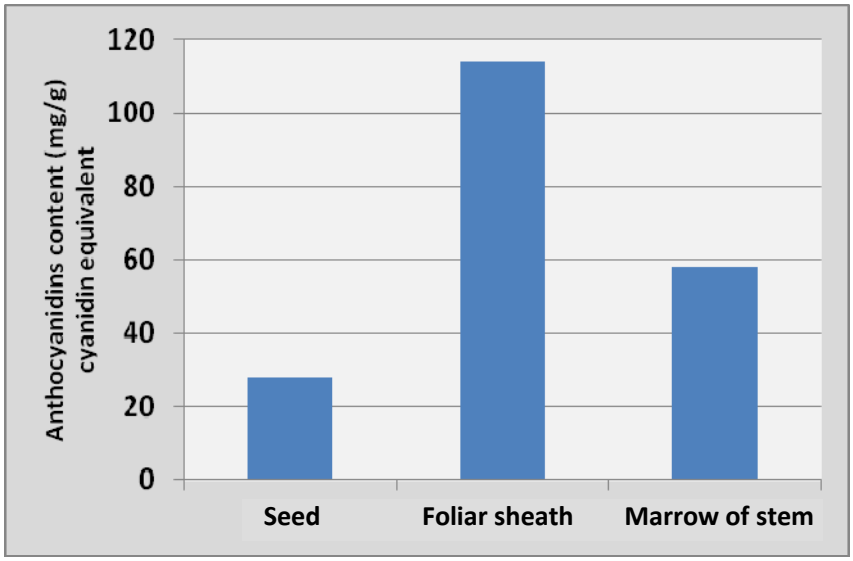


Figure 3. *Anthocyanidins content of different parts of* ***Sorghum caudatum***

Table 4. *TLC data of anthocyanidins extracts of different parts of* ***Sorghum caudatum***

R_f	Seed	Foliar sheath	Marrow of stem
R_{f1} and relative abondance	0.96 (s)	0.88 (S)	0.92 (S)
R_{f2} and relative abondance	-	0.29 (m)	0.29 (s)

s - small; S - strong; m – middle; R_f - frontal report.

Flavonic aglycones

The flavonic aglycones obtained after the complete hydrolysis of all glycosidic bonds present in the molecules correspond to the skeletons of the flavonoids present in the extracts. The results of proportioning, expressed in quercetol equivalents, are presented in Figure 5.

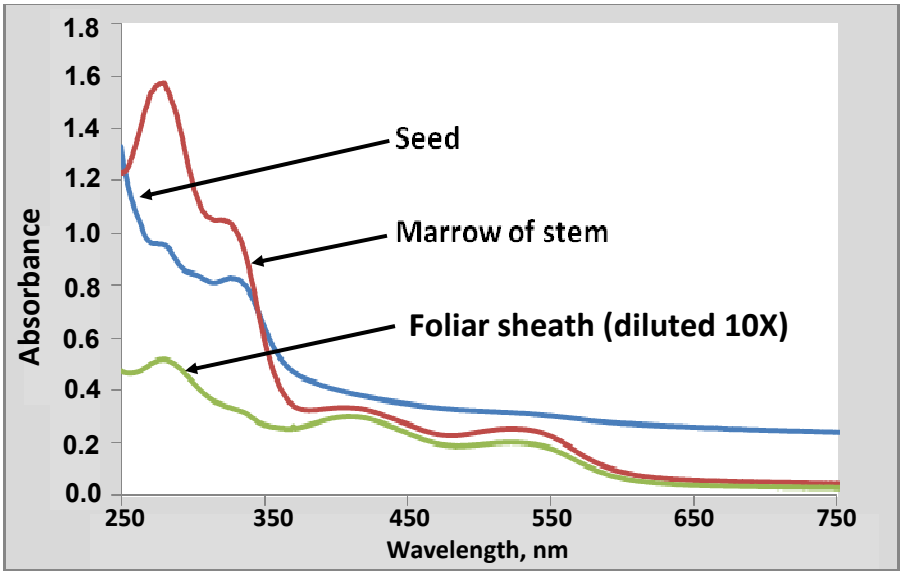


Figure 4. *UV-Visible spectra of rough extracts*

The examination of the R_f (Table 5) reveals the presence of two types of flavonic aglycones almost identical in the various bodies of the plant but more concentrated in the foliar sheath. The existence of a third R_f on the level of the marrow of stem indicates the presence of a third type of aglycone which is absent in the other extracts. As the anthocyanidin's concentration, the content of flavonic aglycones is higher in the foliar sheath than in the other parts of the plants.

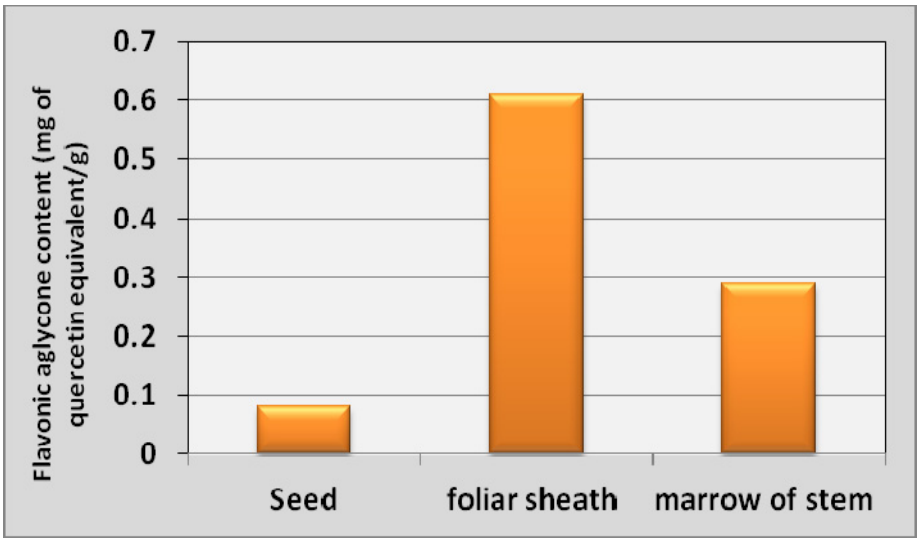


Figure 5. *Flavonic aglycones of different parts of* ***Sorghum caudatum***

Table 5. *TLC data of flavonic aglycones extracts of different parts of* ***Sorghum caudatum***

R_f	Seed	Foliar sheath	Marrow of stem
R_{f1} and relative abondance	0.31 (s)	0.29 (S)	0.27 (s)
R_{f2} and relative abondance	0.77 (m)	0.77 (m)	0.79 (m)
R_{f3} and relative abondance	-	-	0.89 (m)

s - small; S - strong; m – middle; R_f - frontal report.

Anthocyanins

Total anthocyanins

The extracts were initially red in aqueous mediums, and then changed to a dark red color in alkaline solutions and yellow or orange in acidic medium.

These observations indicated the presence of the 3-deoxyanthocyanidins as major component and were in agreement with results from Nip, Gous and other researchers who showed that sorghum anthocyanins are mainly natural 3-deoxy [13-16] that are rare in the vegetable extracts [17]. The appearance of a pink color in marrow of stem extract when the medium become basic confirmed the TLC results and showed the existence of a different type of anthocyanidin in this part of the plant.

Table 6. *Total anthocyanin contents of different parts of* ***Sorghum caudatum***

Parts of the plant / Wine type	$(OD_A - OD_B)$ $\lambda = 520$ nm	Anthocyanins (mg/L)	Anthocyanins (mg/g of dry matter)
Seed of red sorghum	0.008	7	0.35
Marrow of stem of red sorghum	0.168	147	7.35
Foliar sheath of red sorghum	0.244	213.5	10.7
Rosy wine [10]		100	
Red wine of short maceration [10]		200 - 300	
Red wine of 20-25 days of maceration [10]		300 - 800	

The sorghum foliar sheath is exceptionally rich in anthocyanins followed by the marrow of stem. The marrow of stem and foliar sheath extracts (1 g/50 mL) showed anthocyanins concentrations similar to those of the rosy wine and red wine of short maceration (Table 6).

Monomeric anthocyanins

The content of the monomeric anthocyanins was given in aqueous and hydroalcoolic extracts (EtOH : H_2O = 1:1) of the three bodies of the plant (Table 7), in accordance with the method described in literature [45].

Table 7. *Monomeric anthocyanin contents of different parts of* ***Sorghum caudatum***

Parts of the plant	Monomeric anthocyanins (mg 7-O-methylapigenidine/g of dry matter)	
	Aqueous extracts	EtOH : H_2O (1 : 1) extracts
Seed	0.3	2.0
Marrow of stem	3.5	14.3
Foliar sheath	7.6	17.4

Total polyphenols

Six gallic acid solutions with various concentrations (0.8 to 2.5 mg/L) were prepared for the calibration curve (Figure 6). We observed a good correlation between the area of peaks and the concentrations in gallic acid, showing a regression coefficient of 0.995. Using this calibration line, the total polyphenols content was determined in the various parts of the plant and the results were summarized in Table 8.

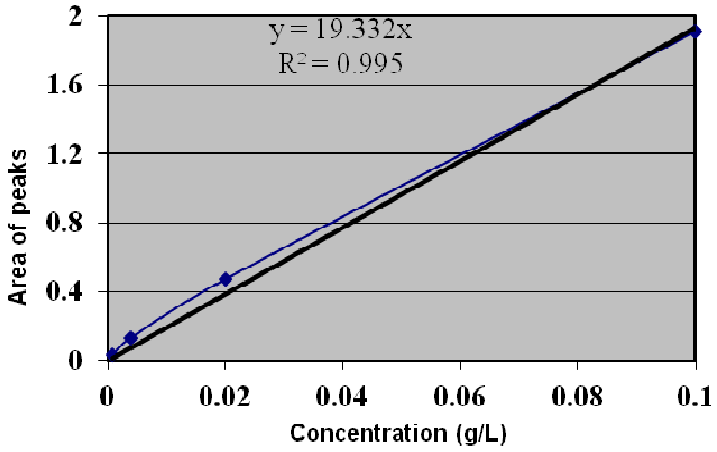


Figure 6. *Calibration curve of gallic acid*

Table 8. *Total polyphenol contents of various parts of sorghum*

Parts of the plant	Concentration (g GAE/L of solution)	Polyphenol content (mg of GAE/g of dry matter)
Seed	0.39	19.8
Marrow of stem	4.67	231.4
Foliar sheath	4.63	233.5

GAE - gallic acid equivalent

According to these results we noted that the marrow of stem and the foliar sheath have very close polyphenols contents. These two bodies contain 11 to 12 times more phenolic compounds than the seed which contains only 20 mg GAE/g.
Table 9 presents the summary of the analysis of the polyphenols content in various parts of Benin red sorghum.

Table 9. *Summary of phenolic compounds content of various parts of red sorghum*

Phenolic compounds	Parts of plant		
	seed	foliar sheath	marrow of stem
Anthocyanidins[a]	28	114	58
Flavonic aglycones[b]	0.082	0.606	0.292
Total anthocyanins[c]	0.35	10.63	7.35
Monomeric anthocyanins[d]	0.3	7.57	3.54
Catechic tannins, mg/g	4.96	71.01	53.55
Total polyphenols[e]	19.78	233.5	231.4

a - mg of cyanidin equivalent /g of dry matter; b - mg of quercetol equivalent/g of dry matter; c - mg of malvidine-3-glucoside equivalent/g of dry matter; d - mg of 7-O-methylapigenidin equivalent/g of dry matter; e - mg of gallic acid equivalent /g of dry matter.

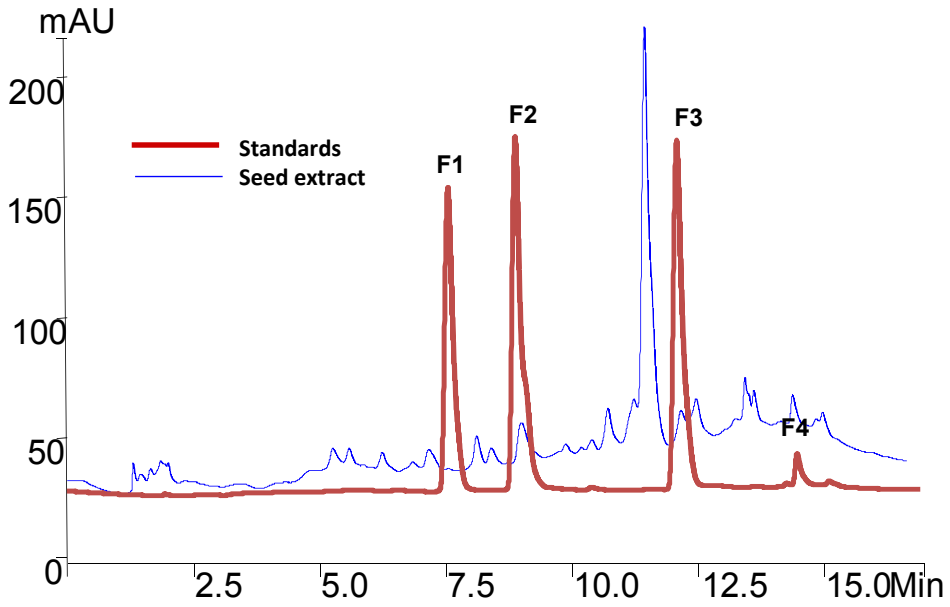
HPLC analysis

The chromatographic analysis of the extracts of the various parts of the sorghum was carried out by HPLC equipped with a UV detector, a pumps-325 system, and an automatic-465 injector. The detection of the peaks is made to 280 nm. A C18 column 5 µm (250 × 4.6 mm) was used. The mobile phase was a binary solvent system: H_2O/HCOOH (1%) and CH_3CN/HCOOH (1%). The gradient of elution is presented in Table 10.

Table 10. *Elution gradient of sorghum polyphenols*

Time (min)	%A (v/v)	%B (v/v)
0	95	5
0.5	95	5
10	90	10
13	75	25
15	50	50
17	95	5

The representative RP-HPLC chromatograms of flavonoids standards and sorghum seed extract are depicted in Figure 7, and those of different plant parts in Figure 8.



F1: Catechin; **F2**: Epicatechin; **F3**: Epigallocatechin; **F4**: Taxifolin

Figure 7. *Representative RP-HPLC chromatograms of flavonoids standards and* ***Sorghum caudatum*** *seed extract*

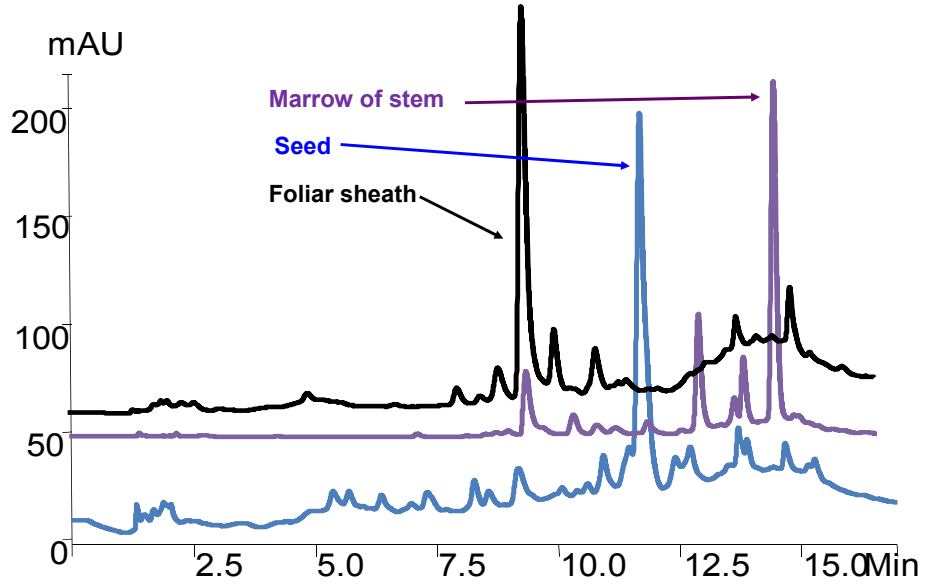


Figure 8. *Representative RP-HPLC chromatograms of different parts of* ***Sorghum caudatum***

DISCUSSION

According to phytochemical screening results we can note that the plant contains a varied range of secondary metabolites in variable concentration in its various parts. It is exceptionally rich in saponins and phenolic compounds particulary in flavonoids: tannins and anthocyanins. The concentration of free quinones, anthracene derivatives, saponins, tannins and anthocyanins is higher in the foliar sheath and in the marrow of stem than that in the seed. The content of catechic tannins is 11.4% in the foliar sheath, slightly higher than that of the red wine; 5.8% in marrow and 2.8% in seed. The saponins, gallic tannins and mucilages are almost absent from seed which contains O-heterosides with reduced genine which is absent from other parts. The positive reaction to the Dragendoff's test and the Lieberman-Burchard reagent confirms the results of Falade *et al.* [43].

The observations about qualitative analysis of anthocyanidins are in agreement with the quantitative results which confirm the richness of the foliar sheath in anthocyanidin (114 mg/g) followed by the marrow of stem (58 mg/g) contrary to the seed which contains only very little of anthocyanidins (28 mg/g). The various values of R_f showed the presence of a particular type of anthocyanidins in each body of the plant. On the other hand the value of the second frontal report (R_{f2}) confirmed the presence of the same type of anthocyanidin in the foliar sheath and in the marrow of stem but more abundant in the foliar sheath. This anthocyanidin is absent in the seed. The UV-Vis spectra (Figure 4) of the three extracts recorded between 250 and 750 nm confirmed these assumptions.

It arises from the analysis of curves in Figure 4 that the foliar sheath, here 10 times diluted, is richer in phenolic compounds than the marrow of stem and seed. The superposition of the curve representing the foliar sheath extract to that representing the marrow of stem extract in the field of the visible light, confirms our chromatographic

(TLC) observations; thus two bodies of the plant would contain the same type of compounds. We can allot the spot of R_f = 0.29 observed into TLC to the family of anthocyanidin which absorbs in the visible light wavelength. The diversity of spectral profiles observed on the level of different extracts in visible light is well in agreement with the variability of chemical composition of these organs which was revealed by the chromatographic observations.

At this stage of our study we note a convergence between the chromatographic results and spectroscopic measurements.

We note, starting from results summarized in Table 7, that the foliar sheath contains twice more monomeric anthocyanins than the marrow of stem which contains ten times more than the seed. The comparison of these reports to those of the total polyphenols content leads us to conclude that the seed of the sorghum contains more phenolic compounds, not anthocyanic, than other bodies of the plant which are richer in anthocyanins. In addition, it is noted that the presence of organic solvent (ethanol) in the extraction medium allows a stronger extraction of the anthocyanins. Indeed, 50% of ethanol in water extracts two times more monomeric anthocyanins than pure water starting from the foliar sheath, and respectively four times and six times more starting from marrow of stem and from the seed.

The comparison of retention times and the extracts with standards co-injection results led us to detect the presence of taxifolin in all three parts, but in higher proportion in the marrow of stem. The catechine fairly present in the foliar sheath is quasi-absent in the other bodies. On the other hand, the epicathechin and the epigallocathechin, clearly identified in seed seem are absent in the marrow of stem and the foliar sheath.

CONCLUSIONS

Despite the Beninese *Sorghum caudatum*'s importance in food, biology and medicine, only the seed of this specie has been studied. The current study demonstrated the richness of red sorghum, especially that of its foliar sheath, in phenolic compounds, natural antioxidants, which have a considerable interest in food and pharmacological industries. This exceptional richness of *Sorghum caudatum* in 3-deoxyanthocyanidins makes it a potential source of alimentary dyes. Our study contributes to increase this plant's phytochemical knowledge and allows having a better understanding of the pharmacological properties of its leaf sheath's extracts.

The composition of *Sorghum caudatum*'s leaf sheath exceptionally rich in polyphenols may be the basis of its use in Benin's pharmacopoeia as antianemic and in food industry as an antioxidant in the stabilization of certain foods such as milk cheese, maize porridge etc.

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