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Quantification of total phenols and flavonoids of *Desmodium adscendens* (Sw.) DC. (Papillionaceae) and projection of their antioxidant capacity

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ABSTRACT

Objective: The present study highlights the potential of *Desmodium adscendens* in the trapping process of toxic oxygen radicals, particularly its flavonoid extracts.

Methodology and results: First, the levels of polyphenols and flavonoids in leaves and stems of *D. adscendens* were evaluated by spectrophotometry. The leaves are richer in total polyphenol (3768.33 µg GAE/g) compared with stems (2153.67 µg GAE/g). There were 0.077% of flavonoids found in leaves; while in the stems were 0.02%. Tests against free radicals of DPPH (1, 1'-diphenyl-2-picrylhydrazyl) were made with different extracts of leaves and stems of *D. adscendens*. The ethyl acetate extract of the leaves (EC_{50}= 0.26 mg/ml) showed the strongest antioxidant activity.

Conclusions and potential application of findings: This study confirms the antioxidant activity of flavonoid extracts reported by various studies. The results of this study can be a rational scientific explanation to the large use of *D. adscendens* in non-conventional medicine by the populations. This study is a contribution to the search for natural antioxidants.

Key words: Polyphenols, flavonoids, *Desmodium adscendens*, antioxidant

INTRODUCTION

Free radicals are chemical species that possess an unpaired electron (single) on their outer orbital layer. They are likely to degrade by oxidation of biological molecules called oxidative stress. Oxidative stress appears to be the main cause of many diseases: cardiovascular diseases, cancer, cataracts, Alzheimer's disease (Petrone *et al*., 1980, Halliwell *et al*., 1992, Finkel and Holbrook, 2000), Parkinson's disease, ulcer (Atawodi, 2005), edema and premature aging of the skin (Georgetti *et al*., 2003). Any substance or molecule antagonist effect with respect to the production of free radicals is known as an antioxidant. Flavonoids have a strong antioxidant potential (Husain *et al*., 1987; Harbone and Williams, 2000; D'abrosca *et al*., 2007). *Desmodium adscendens* (Sw.) DC. a herb with slender branches finely pubescent, more or less prostrate, rooting at the nodes belongs to the family of Papillionaceae. It is a plant species common from Guinea, Cameroon and to Zimbabwe, and it is also in tropical America. It is a sole runway hedge, preferring damp locations and t is found in forested areas and on the edge of savannas (Adjanohoun *et al*., 1984). Previous studies noted the presence of polyphenols and particularly flavonoids in methanolic crude extracts of *D. adscendens* (Mamyrbekova *et al*., 2008). This work aims to

quantify the polyphenols and flavonoids of this plant species and assess the free radical scavenging capacity of flavonoids extracted selective compared to the DPPH. The result will be a projection of the antioxidant potential of *D. adscendens*.

MATERIAL AND METHODS

Material: The plant material constituted of stems and leaves of *Desmodium adscendens*. The different plant parts were collected in March 2008 on the sites of the Universities of Abobo-Adjame and Cocody (Abidjan, Côte d'Ivoire). They were previously identified in accordance with available herbarium at the National Center floristic (CNF) based in the University of Cocody with the help of botanists from this center. These plant parts were rinsed in running water, dried in air continuously for one week and then again dried in an oven (50°C) for 24 h. After drying, the plant parts were ground using an electric grinder (Brand RETSCH, Type SM 100) to obtain fine powders which were used in the preparation of extracts.

Preparation of plant extracts: The total flavonoids extracts were obtained from the crude methanol extracts of leaves (S_1) and stems (S_2) respectively by the method described by Chen *et al.,* (2006) with some modifications. Two hundred grams (200 g) of fine powder of the plant material were mixed in 1l of 70% (v/v) aqueous methanol under permanent agitation for 24 h at ambient temperature. The extract was filtered first through a filter paper and then through cotton wool. The extract was concentrated using a rotary evaporator in a water bath set at 65°C. The aqueous residual was kept for 48 h in a refrigerator then decanted. The aqueous extract was treated successively with hexane (500 ml), chloroform (500 ml), ethyl acetate (500 ml) and n-butanol (500 ml) to give four organic extracts hexane (S_1^I, S_2^I), chloroform (S_1^{II}, S_2^{II}), ethyl acetate (S_1^{III}, S_2^{III}) and n-butanol (S_1^{IV}, S_2^{IV}) containing total flavonoids.

Phytochemical screening: The phytochemical screening of S_1, S_2, S_1^I, S_2^I, S_1^{II}, S_2^{II}, S_1^{III}, S_2^{III}, S_1^{IV}, S_2^V was realized by TLC (silica gel $60F_{254}$ plates, Merck) according to the methods of Markham (1982), Ladiguina (1983) and Lagnika (2004) to confirm the presence of only flavonoids in ethyl acetate (S_1^{III}, S_2^{III}) and n-butanol (S_1^{IV}, S_2^V) extracts.

Dosage of phenolic compounds: The phenolic compounds contents in $S_1$and S_2 were valued slightly according to the method of Singleton and Rossi (1965) with some modification. To 1 ml of $1/10^{th}$ dilute extract was added 1 ml of Na_2CO_3 (17%, m/v) and 5 ml of (0.5 N) Folin-Ciocalteu reagent, incubated at 37°C during 30 min. The absorbance was measured at 760 nm against a blank. The phenolic content was expressed as Gallic acid equivalents using the following linear equation based on the calibration curve:

$Y= 6,8583X$, $R^2= 0, 9593$;

Y is the absorbance and X is the concentration as Gallic acid equivalents. The results are expressed in micrograms equivalent Gallic acid by grams of the powder dry mass of the plant material (µg GAE/g).

Quantification of total flavonoids: The determination of total flavonoids was done by the method of Hariri *et al.*, (1991). 1 g of powder of each plant organ was mixed in 100 ml methanol 70% (v/v). After 24 h, 2 ml of filtrate were mixed with 100 µl of Neu reagent. The absorption was determined at 404 nm and compared to the one of standard quercetin (0.05 mg/ml) treated with the same reagent. The percentage of total flavonoids is then equivalent quercetol calculated using the formula

$F = (0.05 \times Aext / Aq) \times 100 / Cext$ (%),

Aext: Absorption of the extract, Aq: Absorption of quercetol, Cext: Concentration of extracted plant material, or 10 mg/ml

DPPH scavenging assay for determination of antioxidant activity: The radical scavenging essay of extracts S_1, S_2, S_1^{III}, S_2^{III}, S_1^{IV}, S_2^{IV} were determined against 1,1'- diphenyl-2-picrylhydrazyl free radical (DPPH; Carlo-Erba) by UV spectrophotometry at 517 nm by the method previously described (Sanchez-Moreno *et al*., 1999). The following concentrations of 0.5, 0.1, 0.025, 0.01, 0.001 mg/ml were prepared in absolute ethanol. Vitamin C was used as antioxidant standard at concentrations of 0.5, 0.1, 0.025, 0.01, 0.001 mg/ml. 2.5 ml of extract combined with 1 ml of DPPH dissolved in absolute ethanol (0.3 mg/ml) was placed in a test tube.ml 2.5 ml of extract combined with 1 ml of DPPH dissolved in absolute ethanol (0.3 mg/ml) was placed in a test tube. After shaking, the tube was placed safe from light during 30 min. A blank solution was prepared containing the same amount of ethanol and DPPH. The radical scavenging activity was calculated using the following formula:

$I= [(A_b - A_e) / A_b] \times 100$ where I is the percentage of inhibition, A_b absorbance of the blank sample and A_e is the absorbance of the extract.

Statistical analysis: All tests were done in triplicate. Data were expressed as means ± standard errors. EC_{50} were determined graphically.

RESULTS

Phytochemical screening: Phytochemical screening of extracts S_1, S_2, S_1^{III}, S_2^{III}, S_1^{IV} and S_2^{IV} revealed the presence of flavonoids by the numerous fluorescent colors of spots observed on the chromatograms under UV at 366 nm (Tables 1-3). Fluorescent orange, red, yellow, blue and green observed with Neu reagent are characteristic of flavonoids. With aluminum chloride, yellow, brown and blue-pale staining was observed, whereas, with Godin reagent, fluorescent yellow, blue and green were observed.

Dosage of phenolic compounds: The test performed on crude extracts was used to determine the total phenolic content of different organs. The results are expressed in micrograms equivalent Gallic acid by grams of the powder dry mass of the plant material (µg GAE/g). The levels of 3768.33 and 2153.67 µg GAE/g were recorded respectively with the leaves and stems of *D. adscendens*. The leaves had therefore the highest amount of phenolic derivatives.

Table 1: TLC analysis of crude extracts (S_1, S_2); gradient (*n*-BuOH/ AcOH /H_2O 4: 1: 5; v/v/v)

Crude extracts	UV/366 nm		$AlCl_3$/ 366 nm		Neu/ 366 nm		Godin/ 366 nm	
	R_f	Color	R_f	Color	R_f	Color	R_f	Color
	0.92	Red[1]	0.90	Red	0.92	Orange[3]	0.92	Red [4]
					0.80	Yellow[3]		
			0.75	Yellow	0.75	Yellow[3]		
S_1	0.72	Yellow[1]					0.72	Yellow[4]
	0.55	Yellow[1]					0.55	Yellow[4]
	0.47	Yellow[1]	0.47	Yellow	0.47	Orange[3]		
	0.35	Yellow[1]			0.35	Yellow[3]		
			0.20	Blue[2]			0.30	Blue
			0.90	Orange	0.92	Orange[3]	0.95	Blue
	0.75	Brown			0.77	Green[3]		
S_2			0.72	Blue[2]			0.72	Yellow[4]
	0.60	Brown	0.61	Yellow	0.61	Yellow[3]	0.63	Purple
					0.44	Orange[3]		
	0.32	Yellow[1]	0.20	Blue[2]	0.35	Yellow[3]		

[1]Flavonoids detected at 366 nm; [2] Flavonoids detected with $AlCl_3$., [3]Flavonoids detected with Godin reagent; [4]Flavonoids detected with Neu reagent

Table 2: Detection of chemical compounds of S_1^{III} and S_2^{III}; gradient ($CHCl_3$/ AcOEt/ AcOH 6: 5: 0.5; v/v/v)

Crude extracts	366 nm		$AlCl_3$ / 366 nm		Godin/ 366 nm		Neu/ 366 nm	
	R_f	Color	R_f	Color	R_f	Color	R_f	Color
					0.94	Orange[3]	0/94	Red[4]
	0.92	Red[1]	0.92	Red	0.92	Red	0.87	Red[4]
	0.62	Red[1]			0.62	Red	0.62	Red[4]
	0.61	Yellow[1]						
	0.54	Purple[1]	0.51	Blue[2]			0.50	Mauve
S_1^{III}			0.46	Yellow				
	0.42	Yellow[1]			0.36		0.38	Yellow[4]
	0.30	Yellow[1]					0.34	Orange[4]
	0.20	Blue-pale[1]					0.23	Blue[4]
	0.11	Purple[1]	0.12	Blue[2]				
	0.07	Yellow[1]	0.07	Yellow			0.07	Green[4]
S_2^{III}	0.92	Blue-pale[1]	0.92	Blue[2]			0.92	Blue[4]

	0.62	Purple[1]					0.62	Mauve
	0.52	Blue-pale[1]	0.52	Blue[2]			0.48	Blue[4]
	0.43	Blue-pale[1]	0.44	Yellow			0.42	Blue[4]
					0.36		0.38	Yellow[4]
	0.35	Purple[1]	0.35	Blue[2]			0.33	Orange[4]
							0.30	Yellow[4]
			0.11	Blue[2]			0.24	Blue[4]
			0.05	Yellow			0.06	Purple
							0.03	Yellow[4]

[1]Flavonoids detected at 366 nm; [2] Flavonoids detected with $AlCl_3$., [3]Flavonoids detected with Godin reagent; [4]Flavonoids detected with Neu reagent

Table 3: Detection of chemical compounds of S_1^{IV} and S_2^{IV}; gradient (n-BuOH/ AcOH/ H_2O 4: 1: 5; v/v/v)

Crude extracts	UV/ 366 nm		$AlCl_3$/ 366 nm		Godin/ 366 nm		Neu/ 366 nm	
	R_f	*Color*	R_f	*Color*	R_f	*Color*	R_f	*Color*
	0.88	Yellow[1]						
	0.81	Brown	0.80	Yellow	0.78	Gray		
	0.74	Yellow[1]			0.74	Yellow[3]	0.74	Yellow[4]
S_1^{IV}	0.64	Brown	0.63	Yellow	0.64	Yellow[3]	0.64	Yellow[4]
			0.54	Yellow	0.61	Gray	0.54	Green[4]
			0.49	Yellow	0.51	Yellow[3]	0.48	Orange[4]
							0.33	Orange[4]
	0.86	Yellow[1]	0.80	Yellow				
					0.79	Yellow[3]	0.77	Yellow[4]
	0.71	Yellow[1]			0.71	Yellow[3]	0.71	Blue[4]
S_2^{IV}	0.63	Brown	0.61	Yellow	0.62	Yellow[3]	0.61	Yellow[4]
					0.59	Gray		
			0.48	Yellow	0.51	Yellow[3]	0.45	Orange[4]

[1]Flavonoids detected at 366 nm; [2] Flavonoids detected with AlCl3.,[3]Flavonoids detected with Godin reagent; [4]Flavonoids detected with Neu reagent

Quantification of total flavonoids: The different rates of total flavonoids are contained in the histogram below (Figure 1).The leaves show the highest rate of flavonoids.

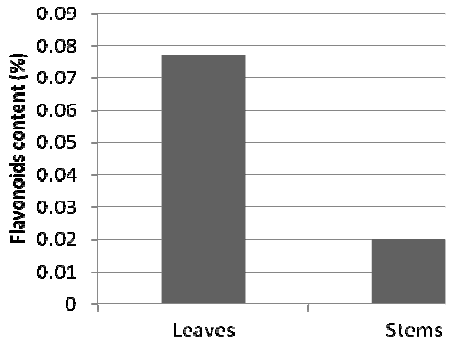

Figure 1: Different levels of total flavonoids

Radical scavenging (antioxidant) activity: The Figure 2 translates the radical scavenging capacity of crude extracts (S_1, S_2), ethyl acetate (S_1^{III}, S_2^{III}) and n-butanol (S_2^{IV}, S_2^{IV}) extracts of the leaves and stems of *Desmodium adscendens*. The results were expressed in percentage of inhibition of DPPH.

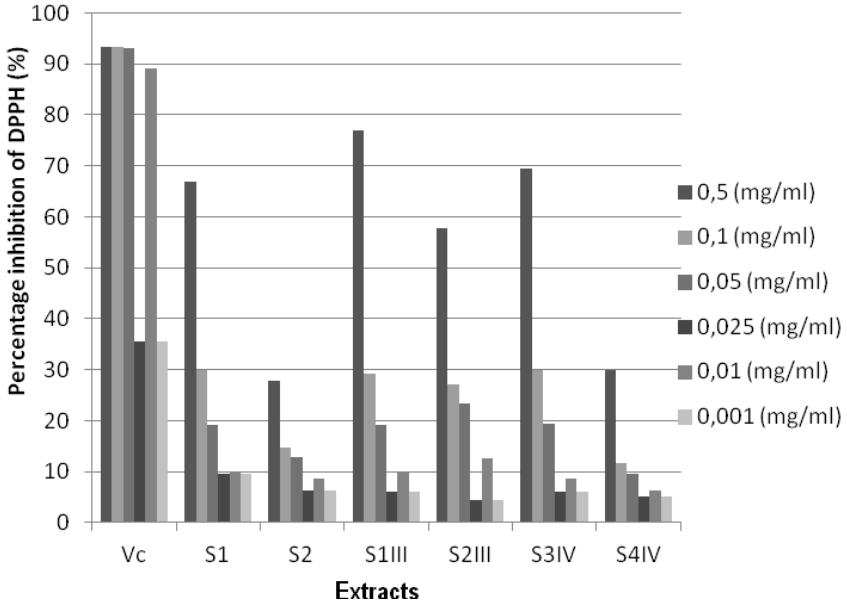

Figure 2: Percentage inhibition of DPPH by S_1, S_2, S_1^{III}, S_2^{III}, S_1^{IV} and S_2^{IV}

We also determined the effective concentrations 50 of different extracts. EC_{50} of 6, 310, 267.5, 387.5 and 293.5 µg/ml were determined for vitamin C, S_1, S_1^{III}, S_2^{III} and S_1^{IV} respectively; fractions S_2 and S_2^{IV} showed an EC_{50} greater than 500 µg/ml.

DISCUSSION

The results of the characterization of flavonoids are reported in Tables 1-3. Flavonoids are fluorescent like other secondary metabolites in UV/366 nm. Indeed, to detect flavonols as yellow and green spots (Rf= 0.75, 0.55, 0.47, 0.35 in S_1, S_2 in Rf= 0.34 Rf = 0.39, 0. 22, 0.07 in S_1^{III}, Rf= 0.74 in S_1^{IV}, Rf= 0.87 in S_2^{IV}), flavones, flavones methylated isoflavones, flavanones and chalcones appear as blue spots (Rf= 0.22 in S_1^{III}, Rf= 0.92, 0.52; in S_2^{III} 0.43), flavones and chalcones have purple spots (Rf= 0.12 in S_1^{III}, Rf= 0.62, 0.35 in S_1^{III}) methylated flavones were found in the form of blue or purple spots (Dawson *et al*., 1991). Markham (1982) and Mohammed (2006) have shown that anthocyanidins-3-glycosides give red spots (Rf= 0.92 in S_1, R= 0.92, 0.62 in S_1^{III}).However, all these fluorescences could also characterize the presence of other secondary metabolites. Therefore, to make sure they belong to the flavonoids, several specific developers were used flavonoids form with several specific reagents ($AlCl_3$, Neu) complexes well colored in the visible or under UV / 366 nm (Georgievskii *et al*., 1990). Indeed, $AlCl_3$ showed the presence of several different spots of fluorescence, which reveals the flavonoids in yellow in the visible and sub UV/366 nm. The spots change color from blue to brown (Rf = 0.20 in S_1, R= 0.72, 0.20 in S_2, R = 0.51, 0.22, 0.12 in S_1^{III}, Rf = 0.92, 0.52, 0.35, 0.11 in S_2^{III}) (Merck, 1980; Lagnika, 2005) or fluorescent yellow-green (Rf= 0.75, 0.47 in S1, S2 in Rf= 0.62 , 0.46, 0.07 in S_1^{III}, Rf = 0.43, 0.52; 0.05 in S_2^{III}, Rf= 0.79, 0.64, 0.54, 0.48 in S_1^{IV}, Rf= 0.80, 0.61, 0.35, 0.47 in S_2^{IV}) (Ladiguina *et al*., 1983).The presence of flavonoids was confirmed by Neu's reagent, which makes them appear as visible as yellow and brown spots (Tables 1-3). Under UV/366 nm observation, those colors are growing and diversifying (Wagner *et al*., 1996). Flavonoids have also been highlighted in UV/366 nm by the appearance of yellow, orange after revelation with Godin's reagent (Chaaib, 2004). Thus, after application of these information from the results obtained (Tables 1-3), a significant presence of

several types flavonoids in the extracts studied (more than 10 compounds in S_1, in 9 S_2, 12 in S_1^{III}, 13 in S_2^{III}, 8 in S_1^{IV}, 5 in S_2^{IV}) was found.

This richness in flavonoids of the study's extracts, forced their quantification using the method described by Hariri *et al.,* (1991). Flavonoids are polyphenols, it seemed quite natural also to quantitatively determine these extracts in the study by the method of Singleton and Rossi (1965). In light of the results, a relatively high rate of phenolic secondary metabolites in leaves of *D. adscendens* (3768.33 mg GAE/g) was seen as compared to that of rods (2153.67 mg GAE/g).In addition, the leaves contain more total flavonoids than the stems (Figure 1). The uneven distribution of phenols in different parts of the plant has been highlighted by several authors (Bano *et al,*. 2003; Falleh *et al*., 2006). Plants are sedentary organisms and in their environment, they are subject to a multitude of stress such as abiotic (UV radiation, nutrient deficiencies or excess or toxic minerals) and biotic (microorganisms). Indeed, the content of phenolic compounds in various organs and tissues of a plant species can be a very meaningful indicator of state changes due to different environmental conditions or in response to conditions of treatment. The content of phenolic compounds in any plant organ is also dependent on a strong genetic influence.

The results of the quantification of antioxidant activity by spectrophotometry (Figure 2) show a progressive decrease in the percentage inhibition of DPPH as the range of concentrations of plant extracts decreased. DPPH is therefore sensitive to different extracts in a dose-response relationship.EC_{50}, scientometric parameter introduced by Brand-Williams et al, was used by several research groups to explain their results. It expresses the concentration that causes 50% loss of activity of DPPH (Molyneux, 2004).The EC_{50} values are lower for the ethyl acetate fractions and n-butanol, which reflects a good demonstration of their antioxidant activity. The high antioxidant activity of ethyl acetate and n-butanol extracts is directly related to their flavonoid revealed by TLC (Husain *et al*., 1987; Harborne and Williams, 2000; D'abrosca *et al*., 2007).

CONCLUSION

This study is a contribution to the development of a medicinal plant from Côte d'Ivoire, namely *Desmodium adscendens*. It was to identify flavonoids in its ethyl acetate and n-butanol extracts by means of TLC, to determine the quantity and make a projection on its antioxidant capacity by DPPH spectrophotometric method. The antioxidant activity of extracts of organs of *D. adscendens* revealed would be largely due to the presence of flavonoids in their bodies that were identified by TLC and identified but also quantified by spectrophotometry. The leaves have a relatively high content of polyphenols but particularly in flavonoids compared to stems. The richness in flavonoids bodies of *D. adscendens* could justify its many uses in traditional medicine.

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