

Phytochemicals of the Extracts of Four Medicinal Plants of Côte D'ivoire and Assessment of their Potential Antioxidant by Thin Layer Chromatography

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Abstract

In this work, we reported the results of the phytochemical studies and the assessment of the potential antioxidant in TLC with DPPH that we realized on the crude extracts of stems leaves of *Adenia lobata* and *Desmodium ascendes*; of roots, leaves of

Glyphea brevis and *Palisota hirsuta*. These plants are used extensively in Côte d'Ivoire to take care of some diseases. The reactions of characterization and the TLC served for the setting in evidence of polyphenols, flavonoids, tannins, coumarins, sterols, terpenoids, reducing compounds, proteins, alkaloids and saponins. The studied crude extracts showed an antioxidant/antiradical activity opposite the DPPH.

Keywords: Medicinal plant; phytochemistry; DPPH; TLC; antioxidant activity

1. Introduction

An antioxidant is a molecule that decreases or inhibits the oxidation of other substances. As can define one him as being a << trapper >> of free radicals (FR), that means all composed susceptible to capture a free electron [1]. The FR play an important role in stress oxidizing (SO). The SO appears when the antioxidant/pro-oxidizing balance is broken; he follows an overproduction of RL then [2-3]. He is recognized to be responsible for numerous pathologies: the Parkinsonism, Alzheimer's illness, cardiovascular illnesses, cancers, mongolism, atherosclerosis, arthritis, diabetes, asthma, neurodegeneration, rheumatism, precocious ageing ... [4-8].

The interest carried these last decades to the antioxidant molecules, don't stop growing. Indeed, many works among others [2-19], return the antioxidant activity of the natural substances more and more (NS) of plant origin. The NS are studied to find new structures models to the ends of the conception of a new generation of medicines.

The four medicinal plants of Côte d'Ivoire chosen for our investigating were never the subject of survey on the antioxidant activity to our knowledge: *Adenia lobata* (Jacq.) Engl. (Passifloraceae) is used extensively in the treatment of icterus, of the cephalous, of the otitis. Of other activities (toxicity, ichthyotoxicity) of this plant are signaled [20]. *Desmodium adscendes* DC. (Papilionaceae) is valued a lot by the folk healers. This plant is used like galactagogue, antipyretic and aphrodisiac [20]. *Glyphea brevis* (Spreng.) Monachino (Tiliaceae) is used against the pains of eyes and throat. This plant species would be prescribed in the barrenness of the couple [20]. *Palisota hirsuta* (Thunb.) K. Schum. (Commelinaceae) is prescribed against gonorrhoea, the whitlows, the adenitis, the articular pains, and worm of guinea. As one signals that this plant would be haemostatic and aphrodisiac [20].

The present survey was about the research of the phytochemical compounds and on the assessment of the antioxidant potential of the crud extracts of the organs (stem, root, leaf) of the plants that we selected. For it, our objectives were the next one:

- Characterization by colorful reactions and by thin layer chromatography (TLC), of the different phytochemical constituents that our plants contain;
- assessment by TLC with 1,1-diphenyl-2-picrylhydrazyl (DPPH) of their antioxidant potential.

2. Material and Methods

2.1. Plant Material

It is constituted of stems and leaves of *A. lobata*, *D. adscendes*; of roots and leaves of *G. brevis* and *P. hirsuta*. These different organs were harvested in March 2008 on the sites of the Universities Abobo-Adjame and Abidjan-Cocody, in Côte d'Ivoire. They were identified previously by botanists of the Centre National Floristique (CNF) suited to the university Abidjan-Cocody. These organs were washed to the current water, dried under permanent air-conditioning during one week, then again dried in steam room (50°C) during 24 h. After drying, the organs were pulverized with an electric grinder (Mark RETSCH, Type SM 100) to get fine powders with served for the preparation of extracts.

2.2. Preparation of Extracts

Different types of extracts were obtained from fine powders. Maceration was made with 200 g of powder in 3 x 1000 mL of MeOH-H₂O (70:30, v/v), placed under agitation during 24 h. After filtration, the extracts are concentrated with a rotavapor and kept at freezer during 48 h to precipitate the vegetable fat [21]. After filtration, the solutions served for phytochemical and antioxidant studies.

2.3. Phytochemical Study

The principal phytochemical constituents were characterized in AdF, AdT, DeF, DeT, GIF, GIR, PaF, with colorful reactions and by the establishment of their chromatographic profiles by TLC.

2.3.1. Characterization of the Principal Phytochemical Constituents

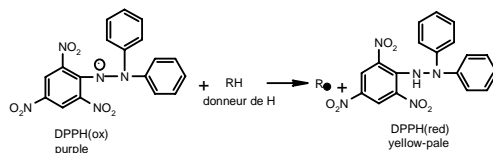
The usual reagents of characterization that we used, allowed us to put in evidence of the groups of following chemical compounds: polyphenols (FeCl₃, 2%), flavonoids (NH₄OH, Chinoda's test, boric acid, acetate of lead, cyanidine), tannins (Stiasny's test, gelatin), gallic tannins (NaNO₃), catechic tannins (aqueous bromine), coumarins (test on the cycle lactonique, in the KOH), sterols and triterpens (Liebermann-Büchard), reducing sugars (Fehling and Tollens), proteins (biuret), alkaloids (Dragendorff's, Burchard's, Wagner's reagents and picric acid), saponins (determination of indicator moss, Im) [22-25].

2.3.2. Thin layer Chromatography

We worked with the crude extracts of AdF, AdT, DeF, GIF, GIR, PaF and Par. An aliquot of every extract is dissolved in 1 mL of appropriate solvent (generally CHCl₃). For TLC, we used silica gel sheets (silufol 60 F₂₅₄, aluminum support; Merck) in appropriate solvent system: CHCl₃/MeOH/AcOH (18:1:1, v/v/v), revealing - reagent's Dragendorff for alkaloids; n-BuOH/AcOH/H₂O (4:1:5, v/v/v), revealing - acetate of lead (5%) and alcoholic KOH (5%) for coumarins; n-BuOH/AcOH/H₂O (4:1:5, v/v/v), revealing - AlCl₃ (0,5 g/100 mL of EtOH) and Neu's reagent for flavonoids; n-BuOH/AcOH/H₂O (4:1:5, v/v/v), revealing - FeCl₃ (10% in MeOH/H₂O, 1:1, v/v) for tannins; cyclohexane/AcOEt (10:1, v/v), revealing - Liebermann-Büchard's reagent for sterols and triterpenes. R_f was calculated for every constituent.

2.4. Antioxidant Test

The chemical test used to value the potential antioxidant activity of extracts by means of TLC, is based on the method of Takao *et al.*, [26]. The detection of this activity is founded on the principle of capture of FR provided by DPPH. When DPPH is reduced by a donor of hydrogen, its original purple color turns to the yellow-pale.



The rapid evaluation of antioxidant activity was determined as previously described [27]. The silica gel sheets were allowed to dry and spray with 0,5% solution of DPPH (Scharlau Chemie) in MeOH. After optimal time of reaction (30 min), the antioxidant activity appears as yellow-pale stains on purple bottom.

3. Results and Discussion

3.1. Main Phytochemicals of the Crude Extracts

3.1.1. Reactions of Characterization

The phytochemical screening achieved on the crude extracts of the organs of *A. lobata*, *D. adscendes*, *G. brevis* and *P. hirsuta* gave the results consigned in the Fig.1. This survey revealed the presence of polyphenols, flavonoids, sterols, triterpens, reducing sugars in all extracts. Gallic tannins are present in 5 extracts (AdF, DeF, DeT, GIF, GIR) whereas catechic tannins were discovered in 7 extracts (AdT; DeF, DeT; GIF, GIR; PaF, PaR). Coumarins are present in 7 extracts except in GIR. Four extracts: AdF, GIR, PaF and PaR showed a presence of proteins while these last are absent in AdT, DeF, DeT and in GIF. The traces of alkaloids were discovered in 7 extracts except in one only, AdF. Steroidic saponins are present in 3 extracts, DeF, DeT and PaF. On the other hand, their respective Im 167, 167, 100 demonstrate that these organs are not considerable sources of saponins. The reactions of characterization of the anthracenic derivatives, cardiac glycosides and quinones were negative on all samples.

The phytochemical screening of *P. hirsuta*, of stems of *A. lobata*, of roots of *G. brevis* and stems of *D. adscendes* was achieved for the first time. On the other hand, Bouquet and Debray [20] published already that the leaves of *G. brevis* contain alkaloids were precipitated by Dragendorff's and Mayer's reagents. Quinones are there absent. These results agree with ours obtained (Tableau 1).

Tableau 1: Result of the phytochemical screening by colored reactions of characterization

Extract	Pol	Fla	Tan	Cou	Ste/terp	Ad	Cg	Rs	Pro	Qui	Alc	Sap
AdF	+	+	+ gal	+	+	-	-	+	+	-	-	- Im = 0
AdT	+	+	+ cat	+	+	-	-	+	-	-	+	- Im = 0
DeF	+	+	+ gal cat	+	+	-	-	+	-	-	+	+ Str Im =167
DeT	+	+	+ gal cat	+	+	-	-	+	-	-	+	+ Str Im = 167
GIF	+	+	+ gal cat	+	+	-	-	+	-	-	+	- Im = 0
GIR	+	+	+ gal cat	-	+	-	-	+	+	-	+	- Im = 0
PaF	+	+	+ cat	+	+	-	-	+	+	-	+	+ Str Im = 100
PaR	+	+	+ cat	+	+	-	-	+	+	-	+	- Im = 0

Presence: +; absence: -

Pol: polyphenols; **Fla:** flavonoids; **Tan:** tannins; **gal:** gallic; **cat:** catechic; **Cou:** coumarins; **Ste/terp:** sterols/triterpenes; **Ad:** anthracenic derived; **Cg:** cardiogenic glycosides; **Rs:** reducing sugar; **Pro:** proteins; **Qui:** quinones; **Alc:** alkaloids; **Sap:** saponins; **Im:** indicator moss; **Str:** steroidic; **AdF:** extract of leaves of *Adenia lobata*; **AdT:** extract of stem of *Adenia lobata*; **DeF:** extract of leaves of *Desmodium ascendens*; **DeT:** extract of roots of *Desmodium ascendens*; **GIF:** extract of leaves of *Glyphea brevis*; **GIR:** extract of roots of *Glyphea brevis*; **PaF:** extract of leaves of *Palisota hirsuta*; **PaR:** extract of roots of *Palisota hirsuta*

However, these authors could not achieve the tests of characterization of saponins, tannins, and sterols. With regard to the leaves of *A. lobata*, Bouquet and Debray [20] reported that alkaloids (Dragendorff, Mayer) and saponins are there present. While comparing these results with those that we obtained (Fig.1), it appears a divergence. Indeed, the presence of saponins was determined quantitatively by Im using method of [28]. Is the presence of saponins confirmed in the drug so Im ≥ 100 . In our case, Im = 0. What confirms the absence of saponins in AdF. Otherwise, as for the leaves of *D. adscendes*, Bouquet and Debray [20] mention that they don't contain saponins. However, we demonstrated that these organs contain some (Fig. 1).

3.1.2. Thin layer Chromatography

For the identification of the alkaloids, the solvent system CHC₁₃/MeOH/AcOH (18:1:1, v/v/v) was used. The silica gel sheets were revealed by the reagent of Dragendorff. The apparition of orange spotlights certified their presence in all our samples of survey [28-30]. We identified coumarins in all our extracts according to the method described by Wagner and Blatt [29, 30]. Indeed, their presence has been proven by several fluorescent green stains under UV to 366 nm after revelation with 5% of solution of acetate of lead (Fig. 2). Otherwise, these results have been confirmed by the apparition of

yellow stains while dealing the TLC with 5% of a methanol solution of KOH (Fig. 3) [31]. The present of coumarins in our extracts would have a structural basis of daphnetine (yellow fluorescence) [28].

Tableau 2: Detection of coumarins by TLC; solvent system: n-BuOH /AcOH /H₂O (4:1: 5, v/v/v); revealing: acetate of lead (5%); all stains are green under UV366 nm

AdF/R _f	AdT/R _f	DeF/R _f	DeT/R _f	GIF/R _f	PaF/R _f	PaR/R _f
0,26	30	0,77	0,28	0,26	0,76	0,31
0,56	87			0,83		0,77
0,81						
0,86	0,					

Tableau 3: Detection of coumarins by TLC; solvent system: n-BuOH/AcOH/H₂O (4:1:5, v/v/v); revealing KOH (5%); all stains are yellow under UV 366 nm

AdF/R _f	AdT/R _f	DeF/R _f	DeT/R _f	GIF/R _f	PaF/R _f	PaR/R _f
0,26	0,25	0,47	0,72	0,27	0,70	0,25
0,47	0,68	0,70		0,36		0,85
0,59		0,77		0,55		
0,80				0,75		

With regard to the flavonoids, we characterized them with a specific reagent, AlCl₃. Several different fluorescence stains were observed on the TLC, after revelation then visualization under UV 366 nm (Fig. 4) [31, 32]. For every specific spot of color with R_f, we made an assignment with a type of compound, using method described by Markham [33] and used by Mohammedi [34] that we modified.

Tableau 4: Detection of flavonoids by TLC; solvent system: n-BuOH /AcOH/ H₂O (4:1: 5; v/v/v)

Extract	UV 366nm		AlCl ₃ /UV 366 nm		Reagent of Neu UV 366 nm		Type of Phenol/ Possible Flavonoid
	R _f	color	R _f	color	R _f	color	
AdF	0,93	red		orange	0,93	orange	anthocyanidine-3-glycosides Phenolic acid flavonols, flavones, isoflavones, flavonones flavonols
	0,87	blue-white fluor*	0,87	orange			
	0,78	blue-white fluor*	0,78	blue-white			
	0,37	yellow	0,37	blue			
			0,72	blue-white	0,67	yellow	
		0,65	yellow	0,57	yellow		
		0,55	yellow	0,43	yellow		
		0,20	blue-white				
AdT	0,90	blue-white fluor*	0,90	blue			flavonols, flavones, isoflavones, flavonones flavonols
	0,34	yellow			0,34	orange	
			0,76	blue-white	0,76	yellow -orange	
			0,61	yellow	0,61	yellow -orange	
			0,47	yellow	0,47	orange	
			0,34	yellow	0,72	yellow -orange	
		0,24	blue				
DeF	0,91	red	0,91	red			anthocyanidine-3-glycosides flavonols, flavones, isoflavones, flavonones, chalcones flavonols
	0,60	dark purple	0,75	yellow	0,75	yellow	
	0,48	yellow -pale	0,60	yellow			
	0,72	yellow	0,48	yellow -pale			
	0,55	yellow -pale	0,39	yellow -pale	0,93	orange	
		0,20	blue-green	0,80	yellow		
				0,45	orange		
DeT	0,90	orange	0,90	orange			anthocyanidine-3-glycosides Phenolic acid flavonols, flavones, isoflavones, flavonones, chalcones
	0,74	blue	0,74	blue			
	0,60	dark purple	0,60	yellow	0,60	yellow	
	0,68	yellow	0,48	yellow -pale	0,92	orange	
	0,32	yellow	0,20	blue-white	0,77	green	
				0,44	orange		
				0,35	yellow		
GIF	0,90	red	0,90	red			anthocyanidine-3-glycosides flavonols, flavones, isoflavones, flavonones flavonols, flavones, isoflavones, flavonones anthocyanidine-3-glycosides
	0,84	blue-white fluor*	0,84	blue			
	0,72	blue fluor*	0,72	blue			
	0,37	red			0,37	orange	
	0,68	Green	0,58	blue-white	0,92	orange	
	0,56	blue fluor*	0,42	yellow	0,80	green	
			0,30	yellow	0,73	blue	
		0,20	blue-white	0,60	blue		
GIR	0,63	yellow	0,63	blue			flavonols
	0,84	blue fluor*	0,26	blue	0,88	blue	
					0,33	purple	
PaF	0,70	blue	0,70	blue	0,70	green	Phenolic acid
	0,91	blue	0,24	blue	0,80	blue	
	0,84	blue					
	0,40	yellow - green			0,57	orange	
				0,47	blue		
				0,36	yellow -pale		
PaR	0,91	blue-white	0,90	blue	0,90	blue	flavonols, flavones, isoflavones, flavonones
	0,85	yellow	0,73	blue- yellow	0,73	yellow – green	
	0,76	blue fluor*	0,41	blue- yellow	0,82	green	
	0,35	yellow					

*Fluorescent

Besides the reactions of characterization, tannins were put in evidence by TLC (Fig. 1). Under visualization in light of the day, tannins appear as gray spotlights as the literature report it [35].

Figure 1: Chromatographic Profile of characterization of tannins in all crude extracts. Revealing- FeCl_3 (10% in $\text{MeOH}/\text{H}_2\text{O}$, 1:1, v/v); visualization in light of the day. Tannins are revealed as stains makes grays. 1= AdF; 2= AdT; 3= DeF; 4= DeT; 5= GIF; 6= GIR; 7= PaF; 8= PaR.



The revelation of different TLC of the extracts with the reagent of Liebermann-Büchard, confirmed the presence of sterols and triterpens. Indeed, the extracts which showed under UV 366 nm yellow stains, contain some sterols whereas those that presented red stains contain triterpens of type oleanane and ursane (Fig. 5) [36].

Tableau 5: Detection of sterols and triterpens by TLC; solvent system: cyclohexane/AcOEt (10:1; v/v); revealing reagent of Liebermann-Büchard then visualization under UV 366 nm; the yellow and red spotlights correspond respectively to the steroids and to the triterpens of type oleanane and ursane

AdF		AdT		DeF		DeT	
Color	R _f	Color	R _f	Color	R _f	Color	R _f
yellow	0,06	yellow	0,15	red	0,62	yellow	0,14
red	0,95	yellow	0,21	red	0,91	yellow	0,25
		yellow	0,26			red	0,90
		red	0,44				
GIF		GIR		PaF		PaR	
red	0,73	yellow	0,12	red	0,61	Red	0,63
		red	0,80				

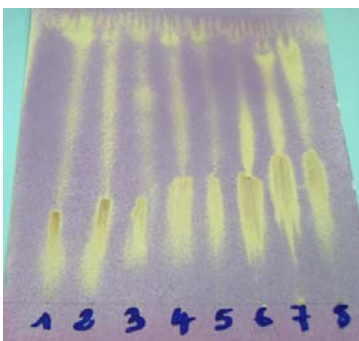
3.2. Dpph test on Tlc

The activity of the phytochemical compounds pulled our attention for their potential role in the prevention of human illnesses. The phenolic compounds are considered like being a major group to the number of the secondary metabolites that contributes to the antioxidant activity of the plants.

Concerning the test of the antioxidant activity achieved for the first time on TLC, all our samples reacted positively with regard to the DPPH (Fig.2). This antioxidant activity is probably due to the presence of the phenolic compounds to the number of which flavonoids and tannins contained in all our extracts [22; 37].

Otherwise, the figure. 2 allow us to observe a superposition of yellow-pale stains (zones of antioxidant activity) and brown grayish under purple bottom. To our opinion, the grayish brown stains are those that correspond to the tannins that we identified (Fig. 1) and that obviously, are inactive with regard to the DPPH.

Figure 2: Chromatographic Profile of antioxidant activity of all extracts. The yellow stains on mauve bottom put in evidence the potential antioxidant activity of extracts 1= AdF; 2= AdT; 3= DeF; 4= DeT; 5= GIF; 6= GIR; 7= PaF; 8= PaR.



4. Conclusion

Adenia lobata, *Desmodium adscendes*, *Glyphea brevis* and *Palisota hirsuta* appear rich in secondary metabolites. It is why; these plants are used extensively in traditional medicine in Côte d'Ivoire to fight and to heal different current affections. The demonstration of the antioxidant activity of the extracts of their organs opposite the radical DPPH, validate the traditional method of their use.

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