Phytochemical screening, evaluation and comparative study of the antioxidant activities of leaves and stem bark extracts from *Anogeissus leiocarpus*: a plant used in traditional medicine in northern ivory coast

Abstract

Anogeissus leiocarpus is a medicinal plant from Ivory Coast. It would be traditionally used to treat several pathologies. The aim of this study was to confirm or refute the use of both leaves and stem bark in the prevention or treatment of diseases linked to oxidative stress. Phytochemical sorting in these two organs of *Anogeissus leiocarpus* showed the presence of flavonoids, coumarins, tannins, phenolic acids, sterols and terpenes. The radical DPPH scavenging activity was carried out qualitatively and quantitatively by thin layer chromatographic (TLC) and spectroscopic methods, respectively. Quantitative analysis by spectrophotometry showed that the antioxidant power of the stem bark is slightly higher than leaves. These organs could therefore be used as good antioxidants in the prevention and treatment of diseases linked to oxidative stress. However, works on safeness should contribute to the valorisation of these materials for giving in use as herbal drug accessible to all social classes.

Key words:*Anogeissus leiocarpus*, phytochemical screening, antioxidant activity, Ivory Coast.

1. INTRODUCTION

For a long time, plants have occupied an important place in human life. All ancient civilizations used plants, either wild or cultivated, for food, defense and medicine [1]. The use of traditional medicine is a very common practice in the countryside and even in the cities. According to the World Health Organization (WHO), 80% of populations in developing countries use medicinal plants to deal with several diseases and provide health care [2, 3]. This situation is explained by the toxicity of synthetic products, the resistance of certain pathogens, the high cost of chemical drugs and the insufficiency of health centers, especially in rural areas [4]. For intance, one of synthetic antioxidants (benzyl benzoate) have proven effective in treating problems related to oxidative stress among this type of compounds somes have presented subsequent consequences and undesirable side-effects [8]. Otherwise, diseases linked to oxidative ~~stess~~ stresssuch as inflammatory conditions, cancers, diabetes, accelerated ageing and cardiovascular diseases constitute public health problems [5, 6, 7]. Faced with this situation, bioactive molecules in herbal medicine have received an increased attention [9 ; 10]. It is in this context that *Anogeissus leiocarpus*, a plant commonly used in traditional medicine in the north of Côte d'Ivoire has been ~~choosen~~ chosenfor a study. The aim of this study was to confirm or refute the use of both leaves and stem bark of *A. leiocarpus* by their ~~antioydant~~ antioxidantactivity in preventing and managing diseases linked to oxidative stress. sSpecifically, it will be to carry out phytochemical screening and evaluate the antioxidant activity of the two plant materials of *A. leiocarpus*.

Comment [MU1]: rewrite

2. MATERIALS AND METHODS

2.1. Material

2.1.1. Plant material

Anogeissus leiocarpus was selected from an ethnobotanical survey carried out among several traditional therapists from north of Ivory Coast. The identification was carried out by botanists

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from Peleforo GON COULIBALY University. Bboth leaves and stem bark were harvested in October 2022 at Peleforo GON COULIBALY university of Korhogo (9° 27' 28" North, 5° 37' 46" West). Samples were washed under running water and dehydrated at room temperature for 10 days, protected from sun light. The dry plant material was then powdered in a mortar and sieved.

2.1.2. Laboratory materials and equipment

The laboratory equipment consists of the usual glassware, an electronic balance (DENVER INSTRUMENT SI-234), a water bath (Neo-Tech SA), a hot plate (Rommelsbcher), an oven (Memmert) and a spectrophotometer (JENWAY 7315).

2.1.3. Reagents and chemicals

The analytical grade chemicals used were purchased from Polychemin (Côte d'Ivoire). For the Thin Layer Chromatography (TLC) tests, silica gel chromatoplates 60 F254, on an aluminum support, were used. The developers and reagents used are 2% $FeCl_3$, 5% KOH, 1% $AlCl_3$, sulfuric vanillin, Dragendorff's reagent and DPPH.

2.2. Methods

2.2.1. Extractions

Extractive techniques by maceration and decoction of our samples were used to obtain the organic and aqueous extracts, respectively.

2.2.1.1. Aqueous extracts

7 g of powder from each organ are boiled in 70 mL of distilled water up to a temperature of 100°C for 30 min. The operation was repeated 3 times. After filtration, the different filtrates were placed in an oven at 50°C for 3 days. The different dry aqueous extracts of *A. leiocarpus* obtained were used to evaluate the antioxidant activity by spectrophotometry.

2.2.1.2. Ethanol extracts

7 g of powder from each organ (leaves and stem bark) of *A. leiocarpus* were macerated in 70 mL of ethanol for 24 h. The operation was repeated 3 times for each organ. After filtration, the different filtrates were placed in an oven at 50°C for 2 days. These extracts were subsequently used to evaluate the antioxidant activity by spectrophotometry.

2.2.1.3. Hydro-ethanolic extracts

7 g of powder from each *A. leiocarpus* organ were macerated in 70 mL of the binary ethanol/water mixture (80 mL/20 mL) for 24 h. After filtration, the macerates were placed in an oven at 50°C for 2 hours to eliminate the ethanol. The operation was repeated 3 times for each organ. The extract obtained for each organ is stored for 24 h in the refrigerator at 4°C for the precipitation of lipophilic compounds. After decantation and filtration, a quantity of these extracts was completely dried in an oven at 50°C for 2 days and these crude hydro-ethanolic extracts were used to evaluate the antioxidant activity by spectrophotometry. The other quantity was used to prepare the selective extracts.

2.2.1.4. Selective extracts

15 mL of each filtrate from the hydro-ethanolic mixture of leaves and stem bark of *A. leiocarpus* were exhausted by successive fractionations with (3 × 10 mL) hexane (C_6H_{14}), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt). The different selective organic fractions were subsequently concentrated in an oven at 50°C. These concentrates were finally used for phytochemical screening and antioxidant activity by TLC.

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2.2.2. Phytochemical screening by TLC

The detection of secondary metabolites (sterols-polyterpenes, alkaloids, coumarins, flavonoids, tannins and phenolic acids) was carried out using a thin layer chromatographic (TLC) plate tests. The screening by TLC of the selective extracts was carried out following the methods described by Mamyrbékova-Békro et al. [11]. Using capillaries, 2 µL of each selective extract were deposited in the form of a point and spaced 0.5 cm from the two edges of the chromatographic plate. The chromatographic plates are then placed in the TLC chamber containing the migration solvents (mobile phase). After development, the chromatograms were visualized with visible and UV 365 nm developers. The colorings appearing in the form of spots are recorded and the frontal ratios (Rf) calculated.

2.2.3. Estimation of antioxidant power

2.2.3.1. Screening by TLC against DPPH of selective extracts

The evaluation of the antioxidant power by TLC used, is a method describe by Takao et al. [12]. 10 µL of solution of each selective extract were deposited on a chromatoplate (silica gel 60 F254, on aluminum support (Merck)), which is then placed in a TLC chamber saturated with migration solvent (mobile phase). After development, the chromatoplate were dried and then revealed using an ethanolic solution of DPPH (0.2 mg/mL). After 30 min of optimal time, constituents of the extract presenting potential anti-radical activity were revealed in the form of pale yellow spots on a purple background. Thus, the frontal ratios (Rf) associated with the yellow spots are calculated.

2.2.3.2. Evaluation of antioxidant activities of aqueous, ethanolic, and hydro-ethanolic extracts by spectrophotometry compared with vitamine C (positive control)

The evaluation of the antioxidant potential of the extracts was carried out following the Blois method [13]. DPPH was solubilized in absolute ethanol to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of each extract were prepared in ethanol absolute. 2.5 mL of plant extract and 1 mL of ethanolic DPPH solution were introduced into dry, sterile tubes. After shaking, the tubes were incubated in the dark for 30min. The absorbance of the mixture was then measured at 517 nm against a blank consisting of 2.5 mL of pure absolute ethanol and 1 mL of DPPH solution. The result was compared with ascorbic acid (vitamin C) tested under the same conditions. The DPPH inhibition percentages are calculated according to the formula :

$$\mathbf{I(\%) = (A_b - A_s) / A_b \times 100}$$

I (%) : Percentage of inhibition
Ab: Absorbance of blank
As: Absorbance of the sample

The concentrations necessary to scavage 50% (IC_{50}) of radical DPPH were determined on the graphs representing the percentage of radical DPPH inhibition as a function of the concentrations of the extracts or vitamin C.

2.2.4. Statistical analyzes

Statistical analysis was performed using EXCEL 2021 software (version 16.0). It was used to draw the different diagrams and determine the IC_{50} of each extract.

3. RESULTS AND DISCUSSION

3.1. Results

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3.1.1. Yields

Extraction techniques by maceration and decoction made it possible to obtain ethanolic, hydro-ethanolic and aqueous extracts. Thus, the different extraction yields of leaves and stem bark of *A. leiocarpus* were calculated and recorded in Table 1. These values vary between 36.76 ±3.30% and 53.24 ±1.87%.

Table 1. Yields of different extracts from the leaves and stems of *Anogeissus leiocarpus*

	ALL Aq	ALL Et	ALL H-Et	ALS Aq	ALS Et	ALS H-Et
R1	44	41.71	51.4	50.43	40	45.6
R2	38.43	34.71	46.12	54.14	33.43	42.88
R3	41.43	33.86	44.45	55.14	37.71	39.9
Rav	**41.29 ±1.90**	**36.76 ±3.30**	**47.32 ±2.72**	**53.24 ±1.87**	**37.05 ±2.41**	**42.79 ±1.93**

R1: yield 1; R2: yield 2; R3: yield 3; Rav: average yield; AL: A*nogeissus leiocarpus*; L: leaf; S: stem bark; Aq: Aqueous; Et: ethanol; H-Et: hydro-ethanolic

3.1.2. Phytochemical screening by TLC

The identification of the different secondary metabolites was done using migration or mobile phase solvents which are :

- Hexane (C_6H_{14}) / ethyl acetate (AcOEt) (5: 0.375; V/V) for hexane extracts;
- Dichloromethane (CH_2Cl_2) / ethyl acetate (AcOEt) / hexane (C_6H_{14}) (2: 2: 1; V/V/V); (CH_2Cl_2) /AcOEt/ (C_6H_{14}) (3:4:2) (V/V/V) and (CH_2Cl_2) /AcOEt/ CH_3COOH (1:3.5:1) (V/V/V) for the dichloromethane extracts;
- (CH_2Cl_2) /AcOEt/ (C_6H_{14}) (3:4:2) (V/V/V) and (CH_2Cl_2) /AcOEt/ CH_3COOH (1:3.5:1) (V/V/V) for the acetate-ethyl extracts;

The major groups of chemical compound identified in our selective extracts are presented in Tables 2 to 6. Each table include information on the retention factor (Rf), visible and ultraviolet (UV) coloring of identified compounds. Reagents such as sulfuric vanillin, Draggendorff, KOH, $AlCl_3$ and $FeCl_3$ were used to identify sterol-terpenes, alkaloids, coumarins, flavonoids, tannins and phenolic acids, respectively.

Table 2. Visible detection of sterols and terpenes from hexanic extracts, with the mobile phase C6H14/AcOEt (5: 0.375) (V/V).

Extracs	R_f (Color) : Possible compounds
ALL H-Et (Hexane)	0.83 (blue) : **sterol** ; 0.73 (blue) : **sterol** ; 0.65 (purple) : **terpene ;** 0.59 (blue) : **sterol** ; 0.5 (blue) : **sterol** ; 0.39 (blue) : **sterol** ; 0.2 (blue) : **sterol** ; 0.11 (blue) : **sterol** ; 0.08 (blue) : **sterol** ; 0.04 (blue) : **sterol** ; 0.0 (pink) : **terpene**
ALS H-Et (Hexane)	0.43 (blue) : **sterol ;** 0.0 (pink) : **terpene**

Table 3. Visible detection of alkaloids from dichloromethane extracts, with the mobile phase CH_2Cl_2/AcOEt/C_6H_{14} (2 : 2 : 1)(V/V/V)

Extracts	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	**No alkaloids identified**

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ALS H-Et (CH_2Cl_2)	**No alkaloids identified**

Table 4. Visible (a) and UV(b) detections of coumarins from dichloromethane extracts, with the mobile phase CH_2Cl_2/AcOEt/C_6H_{14} (2 : 2 : 1)(V/V/V)

Extraits	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	0.8 (yellow[a]) : **coumarin** ; 0.66 (yellow[a]) : **coumarin**
ALS H-Et (CH_2Cl_2)	0.93 (yellow[a], green[b]) : **coumarin** ; 0.8 (yellow[a], green[b]) : **coumarin**

Table 5.Visible (a) and UV(b) detections of flavonoids from dichloromethane and ethyl acetate extracts, with the mobile phase CH_2Cl_2/AcOEt/ C_6 H_{14} (3 :4 :2) (V/V/V)

Extracs	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	0.90 (green[a]) : **flavonoid ;** 0.84 (green[a], blue[b]) : **flavonoid** ; 0.79 (green[a]) : **flavonoid** ; 0.75 (green[a], blue[b]) : **flavonoid ;** 0.70 (green[a], blue[b]) : **flavonoid** ; 0.58 (yellow[a.b]) : **flavonoid** ; 0.53 (yellow[a.b]) : **flavonoid** ; 0.23 (yellow[a]) : **flavonoid** ; 0.11 (yellow[a]) : **flavonoid** ; 0.06 (yellow[a]) : **flavonoid** ; 0.0 (yellow[a]) : **flavonoid.**
ALS H-Et (CH_2Cl_2)	0.89 (green[a]. blue[b]) : **flavonoid** ; 0.75 (yellow[a]. blue[b]) : **flavonoid** ; 0.63 (blue[a]) : **flavonoid** ; 0.4 (yellow[a]) : **flavonoid.**
ALL H-Et (AcOEt)	0.9 (yellow[a], blue[b]) : **flavonoid** ; 0.73 (yellow[a], blue[b]) : **flavonoid** ; 0.64 (yellow[a]) : **flavonoid** ; 0.6 (yellow[a .b]) : **flavonoid** ; 0.5 (yellow[a.b]) : **flavonoid** ; 0.23 (yellow[a.b]) : **flavonoid** ; 0.16 (yellow[a]) : **flavonoid** ; 0.11 (yellow[a]) : **flavonoid** ; 0.06 (yellow[a]) : **flavonoid** ; 0.0 (yellow[a.b]) : **flavonoid.**
ALS H-Et (AcOEt)	0.9 (yellow[a]. blue[b]) : **flavonoid ;** 0.78 (blue[b]) : f**lavonoid.**

Table 6. Visible detection of tannins and phenolic acids from dichloromethane and ethyl acetate extracts, with the mobile phaseCH2Cl2/AcOEt/CH3COOH (1:3.5:1) (V/V/V)

Extraits	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	0.97 (green) : **phenolic acid** ; 0.86 (gray) : **tanin** ; 0.51 (gray) : **tannin** ; 0.43 (gray) : **tannin ;** 0.31 (gray) : **tannin ;** 0.19 (gray) : **tannin ;** 0.08 (gray) : **tannin ;** 0.0 (gray) : **tannin**
ALS H-Et (CH_2Cl_2)	0.81 (gray): **tannin** ; 0.40 (gray): **tannin ;** 0.32 (gray): **tannin ;** 0.27 (gray): **tannin** ; 0.19 (gray): **tannin** ; 0.12 (gray): **tannin** ; 0.0 (gray): **tannin**
ALL H-Et (AcOEt)	0.59 (green) : **phenolic acid** ; 0.49 (green) : **phenolic acid** ; 0.4 (gray) : **tannin** ; 0.26 (gray) : **tannin** ; 0.2 (gray) : **tannin** ; 0.14 (gray) : **tannin** ; 0.07 (gray) : **tannin** ; 0.0 (gray) : **tanin.**
ALS H-Et (AcOEt)	0.4 (gray): **tannin ;** 0.32 (gray): **tannin ;** 0.27 (gray): **tannin** ; 0.17 (gray): **tannin** ; 0.0 (gray): **tannin**

All results of the qualitative analyse by TLC of the extracts of two plant materials of *A. leiocarpus* are recorded in Table 7. It appears that the leaves and stem barks contain sterols,

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terpenes, coumarins, flavonoids, tannins and phenolic acids. On the other hand, no alkaloid have found in both leaves and stem bark.

Table 7. Summary table of phytochemical screening of secondary metabolites from *A. leiocarpus*

	Sterols	Terpenes	Alkaloids	Coumarins	Flavonoids	Phenolic acids	Tannins
ALL	+	+	-	+	+	+	+
ALS	+	+	-	+	+	+	+

Presence (+) ; absence (-)

3.1.3. Antioxidant activity

3.1.3.1. DPPH• scavenging activity by TLC

Mobile phase used to identify phytocompounds are also used for the antioxidant activity by TLC under the same conditions. Results of the antioxidant activity of extracts respect to the DPPH radical evaluated by TLC are presented in Tables 8, 9, 10 and 11. This activity manifest by the coloration of phytocompounds's spots into yellow. This results displayed that hexane, dichloromethane and ethyl acetate extracts of *A. leiocarpus* have anti-free radical activity.

Table 8. Phytocompounds from hexanic extract with DPPH scavaging activity, with the mobile phase C_6H1_4/AcOEt (5: 0.375) (V/V).

Extraits	R_f (Color) : Possible compounds
ALL H-Et (Hexan)	0.95 (yellow) : UC ; 0.83 (yellow) : **sterol** ; 0.39 (yellow) : **sterol** ; 0.11 (yellow) : **sterol** ;0.0 (yellow) **: terpene**
ALS H-Et (Hexan)	0.95 (yellow) : UC ; 0.85 (yellow) : UC ; 0.43 (yellow) : **sterol** ; 0.38 (yellow) : UC ; 00 (yellow) : **terpene.**

UC: unidentified compound

Table 9. Phytocompounds from dichloromethan extract with DPPH scavaging activity, with mobile phase CH_2Cl_2/AcOEt/C_6H_{14} (2 : 2 : 1) (V/V/V)

Extraits	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	0.99 (yellow) : UC ; 0.9 (yellow) : UC ; 0.86 (yellow) : UC ; 0.8 (yellow) : **coumarin** ; 0.74 (yellow) : UC ; 0.66 (yellow) : **coumarin** ; 0.61 (yellow) : UC ; 0.60(yellow) : UC ; 0.55 (yellow) : UC ; 0.50 (yellow) : UC ; 0.44 (yellow) : UC ; 0.41 (yellow) : UC ; 0.33 (yellow) : UC ; 0.25 (yellow) : UC ; 0.21 (yellow) : UC ; 0.15 (yellow) : UC ; 0.10(yellow) : UC ; 00 (yellow) : UC**.**
ALS H-Et (CH_2Cl_2)	0.99 (yellow) : UC ; 0.93 (yellow) : **coumarin** ; 0.84 (yellow) : UC ; 0.8 (yellow) : **coumarin** ; 0.74 (yellow) : UC ; 0.69 (yellow) : UC ; 0.63 (yellow) : UC ; 0.50 (yellow) : UC ; 0.55 (yellow) : UC ; 0.48 (yellow) : UC ; 0.4 (yellow) : UC ; 0.35 (yellow) : UC ; 0.3 (yellow) : UC ; 0.24 (yellow) : UC ; 0.15 (yellow) : UC ; 0.4 (yellow) : UC ; 00 (yellow) : UC.

UC: unidentified compound

Table 10. Phytocompounds from dichloromethan and ethyl acetate extracts with DPPH scavaging activity, with the mobile phase CH_2Cl_2/AcOEt/ C_6 H_{14} (3 :4 :2) (V/V/V)

Extraits	R_f (Color) : Possible compounds

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ALL H-Et (CH_2Cl_2)	0.96 (yellow) : UC ; 0.9 (yellow) : **flavonoid** ; 0.84 (yellow) : **flavonoid ;** 0.79 (yellow) : **flavonoid** ; 0.70 (yellow) : **flavonoid** ; 0.63 (yellow) : UC ; 0.58 (yellow) : **flavonoid** ; 0.53 (yellow) : **flavonoid** ; 0.43 (yellow) : UC ; 0.34 (yellow) : UC ; 0.26 (yellow) : UC ; 0.23 (yellow) : **flavonoid** ; 0.11 (yellow) : **flavonoid ;** 0.06 (yellow) : **flavonoid** ; 0.0 (yellow) : **flavonoid**
ALS H-Et (CH_2Cl_2)	0.94 (yellow) : UC ; 0.89 (yellow) : **flavonoid** ; 0.8 (yellow) : UC **;** 0.75 (yellow) : **flavonoïde** ; 0.66 (yellow) : UC ; 0.63 (yellow) : **flavonoid ;** 0.54 (yellow) : UC ; 0.45 (yellow) : UC **;** 0.4 (yellow) : **flavonoid** ; 0.31 (yellow) : UC ; 0.25 (yellow) : UC ; 0.19 (yellow) : UC ; 0.14 (yellow) : UC ; 0.08 (yellow) : UC **;** 0.0 (yellow) : UC
ALL H-Et (AcOEt)	0.95 (yellow) : UC ; 0.9 (yellow) : **flavonoid ;** 0.83 (yellow) : UC ; 0.76 (yellow) : UC ; 0.73 (yellow) : **flavonoid** ; 0.64 (yellow) : **flavonoid** ; 0.6 (yellow) : **flavonoid** ; 0.5 (yellow) : **flavonoid ;** 0.46 (jaune) : UC ; 0.4 (jaune) : UC ; 0.34 (yellow) : UC ; 0.28 (yellow) : UC ; 0.23 (yellow) : **flavonoid** ; 0.16 (yellow) : **flavonoid** ; 0.11 (yellow) : **flavonoid** ;0.06 (yellow) : **flavonoid** ; 0.0 (yellow) : **flavonoid**
ALS H-Et (AcOEt)	0.9 (yellow) : **flavonoid** ; 0.83 (yellow : UC ; 0.78 (yellow) : **flavonoid** ; 0.68 (yellow) : UC ; 0.66 (yellow) : UC ; 0.55 (yellow) : UC ; 0.46 (yellow) : UC **;** 0.38 (yellow) : UC ; 0.31 (yellow) : UC; 0.24 (yellow) : UC; 0.18 (yellow) : UC ; 0.09 (yellow) : UC ; 0.0 (yellow) : UC

UC: unidentified compound

Table 11. Phytocompounds from dichloromethan and ethyl acetate extracts with DPPH scavaging activity, with the mobile phase CH_2Cl_2/AcOEt/ CH_3COOH (1 : 3,5 :1) (V/V/V)

Extraits	R_f (Color) : Possible compounds
ALL H-Et (CH_2Cl_2)	0.97 (yellow) : **phenolic acid** ; 0.86 (yellow) : **tannin** ; 0.8 (yellow) : UC ; 0.69 (yellow) : UC ; 0.66 (yellow) : UC ; 0.6 (yellow) : UC ; 0.51 (yellow) : **tanin** ; 0.43 (yellow) : **tanin** ; 0.35 (yellow) : UC ; 0.31 (yellow) : **tannin** ; 0.19 (yellow) : **tannin** ; 0.1 (yellow) : UC ; 0.08 (yellow) : **tannin** ; 0.0 (yellow) : **tannin**
ALS H-Et (CH_2Cl_2)	0.89 (yellow) : UC ; 0.85 (yellow) : UC ; 0.81 (yellow) : **tanin** ; 0.70 (yellow) : UC ; 0.62 (yellow) : UC ; 0.56 (yellow) : UC ; 0.52 (yellow) : UC ; 0.49 (yellow) : UC ; 0.42 (yellow) : UC ; 0.40 (yellow) : **tannin** ; 0.32 (yellow) : **tannin** ; 0.27 (yellow) : **tannin** ; 0.24 (yellow) : UC ; 0.19 (yellow) : **tannin** ; 0.12 (yellow) : **tannin** ; 0.09 (yellow) : UC ; 0.0 (yellow) : **tannin.**
ALL H-Et (AcOEt)	0.96 (yellow) : UC ; 0.85 (yellow) : UC ; 0.78 (yellow) : UC ; 0.65 (yellow) : UC ; 0.68 (yellow) : UC ; 0.59 (yellow) : **phenolic acid** ; 0.49 (yellow) : **phenolic acid** ; 0.4 (yellow) : **tannin** ; 0.35 (yellow) : UC ; 0.26 (yellow) : **tannin** ; 0.2 (yellow) : **tannin** ; 0.14 (yellow) : **tannin** ; 0.07 (yellow) : **tannin** ; 0.0 (yellow) : **tannin**
ALS H-Et (AcOEt)	0.89 (yellow) UC ; 0.85 (yellow) : UC UC ; 0.76 (yellow) : UC ; 0.69 (yellow) : UC ; 0.61 (yellow) : UC ; 0.55 (yellow) : UC ; 0.50 (yellow) : UC ; 0.43 (yellow) : UC ; 0.40 (yellow) : **tannin** ; 0.32 (yellow) : **tannin** ; 0.27 (yellow) : **tannin** ; 0.23 (yellow) : UC ; 0.17 (yellow) : **tannin** ; 0.10 (yellow) : UC ; 0.08 (yellow) : UC ; 0.0 (yellow) : **tannin**

UC: unidentified compound

3.1.3.2. DPPH• scavenging activity by spectrophotometry

3.1.3.2.1. DPPH• scavenging percentages of vitamin C and aqueous, ethanolic hydro-ethanolic crude extracts

Concentrations between C6=0.0625mg/mL and C1=2 mg/mL shown a free-radical DPPH scavenging activity. The different percentages of DPPH• inhibition of aqueous, ethanolic and hydro-ethanolic extracts and vitamin C are represented in Figures 1 and 2. These percentages vary between 04.601 ± 0.116% and 79.340 ± 0.926% for samples and 63.316 ± 0.405% to 84.201 ± 0.116% for vitamin C. *Anogeissus leiocarpus* leaf and stembark could be valuable sources of antioxydant bioactive compounds.

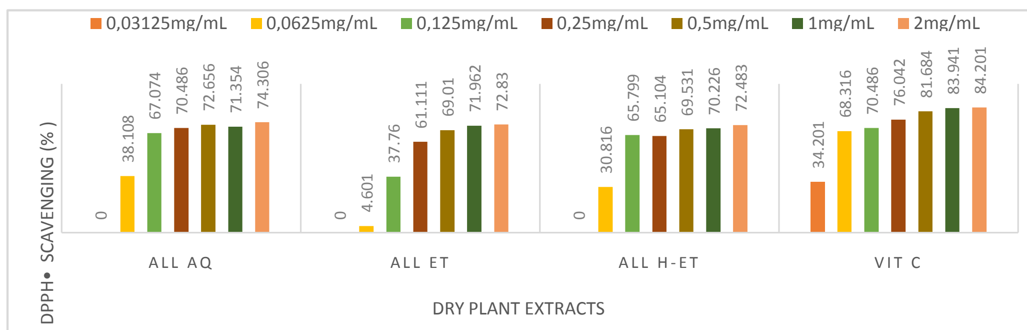

Figure 1. DPPH˙ scavenging percentages of vitamin C and aqueous, ethanolic hydro-ethanolic crude extracts of *A. leiocarpus* leaves

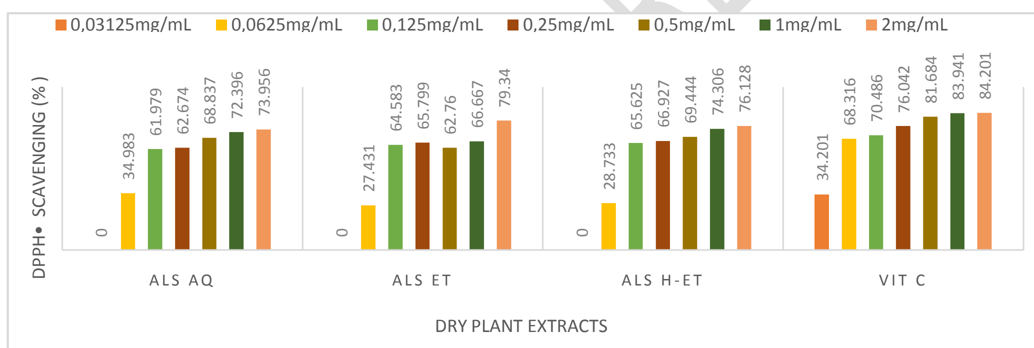

Figure 2. DPPH˙ scavenging percentages of vitamin C and aqueous, ethanolic hydro-ethanolic crude extracts of *A. leiocarpus* stem bark

3.1.3.2.2. Determination of IC_{50} of vitamin C, ethanolic, hydro-ethanolic and aqueous crude extracts

The different inhibition percentages calculated made it possible to determine the concentrations necessary to reduce 50% of DPPH radicals. The IC_{50} was determined graphically using the EXCEL software remplacing y=50 in the equation of line y= ax+b. The average IC_{50} values obtained are recorded in Table 12.

Table 12. IC_{50} values (mg/mL) from extracts

Extracts	Equation of line	IC_{50} (mg/mL)
Vit C	Y= 1091 X + 0.086	**0,04575**
ALL Aq	Y= 462.5 X + 9.202	**0,08822**
ALL Et	Y= 186.81 X + 14.409	**0,19052**

ALL H-Et	Y= 559.73 X – 4.167	**0,09676**
ALS Aq	Y= 431.94 X + 7.987	**0,09727**
ALS Et	Y= 594.43 X – 9.721	**0,10047**
ALS H-Et	Y= 590.32 X – 8.165	**0,09853**

3.2. Discussion

The extractions obtained by decoction and maceration have given yields between 36.76 ±3.30% and 53.24 ±1.87%. The highest yield in the leaves is observed with hydro-ethanolic maceration. Concerning to the stem bark, we observe the highest yield with water decoction. These values are relatively high compared to other study on other plants cited in the literature [14, 15]. These results could justify the current use of water, ethanol and binary water/ethanol extraction methods in a large number of research projects.

Phytochemical screening by TLC allowed the identification of sterols, terpenes, coumarins, flavonoids, tannins and phenolic acids in the two organs of *A. leiocarpus*. On the other hand, alkaloids were absent in the two materials. These results corroborate the work of certain authors. Indeed, Ouédraogo B et al. [16] showed that the stem barks of *A. leiocarpus* are known to be rich in phenolic acids, coumarins, tannins and flavonoids. Yemoa et al. [17] confirmed the absence of alkaloids in these two organs. The presence of these secondary metabolites could justify the use of *A. leiocarpus* in the traditional treatment of pathologies such as urinary schistosomiasis, amoebic dysentery, pain, trypanosomiasis, helminthiasis [18, 19, 20], diarrhea (infantile), hemorrhoidal crises and fever [21, 22]. In addition, these organs could be used to treat diseases linked to oxidative stress such as inflammatory diseases, cancer, diabetes, accelerated aging and cardiovascular diseases. Indeed, these different secondary metabolites detected contain antioxidant properties that have already been proven [23, 24, 25].

Comment [MU2]: please specify

The evaluation of the antioxidant activity was carried out by TLC and spectrophotometry. DPPH scavenging by TLC made it possible to identify the compounds responsible for the antioxidant activity in the two organs studied. The correspondence between the active zones and the phytocompounds responsible for this activity was established by contrasting screening profiles of both antioxidant activity and phytochemical on TLC. Thus, in the hexanic extracts, some of the yellow spots would correspond to the antioxidant activity of sterols and terpenes (Rf: 0.83; 0.11; 0.0 for the leaves / Rf: 0.43; 0.0 for the stems). Similarly, it was deduced that the antioxidant power presented by the dichloromethane and ethyl acetate extracts of the two materials would be due to the presence of coumarins, flavonoids, phenolic acids and tannins. However, several other compound exhibiting antioxidant activity could not be identified.

Concerning to antioxidant activity by spectrophotometry, the objective was to determine the concentrations able to scavenge 50% (IC_{50}) of DPPH free radical [26]. The obtained results are compared with the positive control (vitamine C) tested under the same conditions. Thus, the study shows concentration-dependent activity were observed in all extracts. The percentages of inhibition of extracts simply represent their capacity to trap free radicals unlike the IC_{50} concentration which is an more precise constant in the interpretation of the results. The lower the IC_{50}, the greater the extract's antioxidant activity. The IC_{50} values of aqueous and organic extracts vary between 0.08822 mg/mL and 0.19052 mg/mL while that of vitamin C is 0.04575 mg/mL. By comparison it is noted that IC_{50} (Vit C) < IC_{50} (ALL Aq) < IC_{50} (ALL H-Et) < IC_{50} (ALL Et), therefore the antioxidant power of the aqueous extract of leaves

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is greater than the hydro-ethanolic extract, which in turn is more significant than the ethanolic extract. Likewise, the IC_{50} of the stem bark extracts vary in the same order of magnitude as that of the leaves because IC_{50} (Vit C) < IC_{50} (ALS Aq) < IC_{50} (ALS H-Et) < IC_{50} (ALS Et). However, we notice all extracts have a significant value of IC_{50} close to 0.1 mg/mL, equal to half of vitamine C except the ethanolic extract of the leaves with an IC_{50} of approximately 0.2 mg/mL.

Therefore, it can be concluded that the antioxidant assay of the crude aqueous, ethanolic and hydro-ethanolic extracts of both leaves and stem bark of *A. leiocarpus* revealed a significant capacity to scavenge DPPH free radical.

4. CONCLUSION

The main objective of this study was to confirm or refute the use of both the leaves and stem bark of *Anogeissus leiocarpus* in traditional medicine for disorder linked to oxidative stress. This study consisted of carrying out phytochemical screening and evaluating the antioxidant activity of extracts from both leaves and stem bark of *A. leiocarpus*. The phytochemical screening by TLC highlighted the presence of flavonoids, coumarins, tannins, phenolic acids, sterols and terpenes in the two vegetable materials of *A. leiocarpus*. However, Alkaloids, were no detected from the two organs studied. Radical DPPH scavenging qualitative and quantitative analysis were performed on aqueous, ethanolic and hydro-ethanolic extracts by TLC and spectrophotometer, respectively. It appears, the two vegetable materials have a significant antioxidant profile. In addition, quantitative analyse shown the antioxydant capacity of stem barks is slightly greater than leaves. otherwise, the antioxidant capacities of the two vegetable materials are not negligible compared to vitamin C. Observed antioxidant properties would be due to a synergistic action of the secondary metabolites present in the different extracts. Thus, *Anogeissus leiocarpus* leaf and stem bark could be sources of bioactive compounds, contributing to the valorisation of these materials and their further application in diseases linked to oxidative stress. However, works on safeness should continue for giving in use *A. leiocarpus* as herbal drug accessible to all social classes.

Comment [MU3]: Rephrase the sentence. Reference from : Mouho, Didier G., et al. "Valorisation of Mangifera Indica Crop Biomass Residues." Industrial Crops and Products, 2018 https://doi.org/10.1016/j.indcrop.2018.07.028.

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