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Studies on Phytochemistry and Antioxidant Potential of *Cleome Gynandra* L. (Capparidaceae) Collected from Contrasted Agro-Ecological Zones in Benin

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Abstract

Spider plant (*Cleome gynandra* L.) is one of the most important traditional vegetables in Benin, albeit underutilized. It has a high nutritive value and contains phenolic compounds that are essential in reducing or preventing the occurrence of chronic and infectious diseases. Concurrently, scanty research information on its physicochemical and medicinal potentials is available. Therefore, this study was undertaken to evaluate quantitatively and compare phytochemical and antioxidant properties of acetone, aqueous and hexane extracts of different parts (leaves, stems, seeds) of two C. gynandra provenances collected from contrasted agro-climatic zones in Benin. Total phenols, flavonoïds, flavonols, and other compounds were determined using standard methods. Antioxidant activities were assessed spectrophotometrically against ferric reducing power (FRAP), and DPPH (1, 1- diphenyl-2-picrylhydrazyl) radical scavenging techniques.

Stem extract had the highest concentration of total phenols in aqueous extract (183.307±2.710mgEAG/gMS in Northern Accession; and 117.762±4.240mgEAG/gMS in Southern Accession), while leaves and seeds from both provenances concentrated 164.597±4.501, 112.653±5.597, 25.477±0.785 and 116.277±5.209mgEAG/gMS

respectively in acetone and hexane extracts. Stem and seeds in both provenances contained 108.228±0.069, 84.983±0.000, 76.388±0.000, 39.908±0.045, 10.537±0.069 and 17.554±0.046µgEQ/mg Extract of flavonoïds in acetone, water and hexane respectively. The reducing power of the extracts was significantly higher than that of the standard drugs used in a concentration dependent manner. Plant organs from Northern accession were higher responsive. ANOVA and Kruskal-Wallis tests showed significant differences in total phenol and flavonoïd contents as well as antioxidant activities between plant organs and provenances.

Keywords: antioxidant activity, Cleome gynandra, flavonoïds and polyphenols, natural antioxidant, pathology, pharmacognosy, phytochemical constituents, phytotherapy.

Introduction

Since ancient times, human being relies on the nature to cover basic food, shelter, clothes and also medical needs. Therapeutic uses of the extraordinary plant virtues for the treatment of numerous diseases are very old and have progressed along humankind history. Although a big part of the 20th century has been dedicated to synthetic molecules, research of new active pharmacological agents via screening of natural resources resulted in the discovery of many useful medicines that begins playing a major role in the treatment of a range of diseases and enhancing human health **[1]**. In spite of all advances in modern medicine, ancestral therapeutic traditions perpetuate themselves in Africa where more than 80% of the population continue using traditional phyto-medicine in health caring. This extensively and widespread uses remain relevant to the accessibility and the availability of the traditional medicine in developing countries, as well as the elevated cost and the harmfulness side effects caused by the synthetic compounds.

Natural products are still gaining in interest worldwide. Indeed, with a more reticent public to chemically synthetic products, industrial sectors such as cosmetic, pharmaceutical, agro-alimentary are increasingly returning to those molecules natural produced due to their original chemical and biologic features. Valorisation of natural originated active principles should therefore represent an enormous economic potential with relevance to the different active compounds that they contain: alkaloids, flavonoïds, tannins, saponosides **[2]**. They constitute an inexhaustible reservoir of popular remedies against severe affections, and remain the most efficient source of natural and common products in medicines **[3]**. Hence, they are not only gifted, but also of effective and culinary qualities and high valuable medicinal virtues. Plants are recognized as a marvellous source of medicines, and thousands of plant species are directly used in the therapy. Thus, on 252 essential medicines recorded by the WHO, more than 50% are produced exclusively from medicinal plants **[4]**. Many plant species are capable to produce a high diversity of secondary metabolites differing from those released from the metabolism of basis **[4]**. However, it has been warned the uses of plant product so directly with the biggest prudence as possible since they contain often in spite their therapeutic effects anti-nutritive compounds leading to toxicity **[5]**. Towards the Beninese medicinal plant valorisation, we are interested in a widely-known species in the pharmacopeia, spider plant (*Cleome gynandra* L.).

Poverty and the insufficient supply of nutritious foods are hindrances to an adequate and balanced diet which is essential for health. This affects majority of people in developing countries **[6]**. This issue may be solved in part by promoting consumption of locally available foods like *Cleome gynandra*. Because many of the households frequently consume these vegetables several strategies have been adopted by farmers to meet the rising demand. Leafy vegetables like *Cleome gynandra* are an excellent source of protein, vitamins and minerals, and dietary fibre **[7]** and being familiar and inexpensive these can be used by large segments of the population to

meet essential dietary requirements. Spider plant vegetables are one of the most cost-effective and sustainable solutions to alleviate micronutrient deficiencies, which affect far more people than hunger alone and are widespread in most of sub-Saharan Africa **[8]**.

Spider plant (Cleome gynandra L.) is a multi-purpose wild vegetable, widely distributed in Benin Republic and throughout the Tropics and Subtropics **[9, 10]**. The plant is an erect annual herb with alternate, palmately compound leaves and its petals are white, pink or lilac. It grows as a weed in this part of the Province and is usually gathered from the wild for food and medicine. It is commonly found on wastes land, road sides and on grass lands. It is a multi- branched annual herb growing to a height of 1.5 m **[11]**. The whole plant of C. gynandra has been reported as herbal medicine to treat diseases such as rheumatism, piles, malaria and tumour. The decoction of the leaves and roots are used to relieve headaches, fever and to treat wounds **[12, 13]**. Consequently, the qualitative screening of phytochemical and antioxidant properties of C. gynandra has been reported in India and other countries **[4, 12, 14, 15]**. **[16]** reported in Benin analeptic, anti-infectious and anti-worm properties of African spider plant and its uses in local diets.

Herbal drugs are the oldest forms of treatments known to the world. Different cultures have used herbs throughout history. Chemical constituents of herbs have high therapeutic value. Most of the prescribed drugs contain plant extracts or active substances obtained from the plant extracts. *Cleome* is a genus of flowering plants in the family Capparidaceae. The genus includes about 170 species of herbaceous annual or perennial plants and shrubs. It has a sub-cosmopolitan distribution throughout the tropical and warm temperate regions of the world **[17, 18]**. The antihyperglycemic activities of different extracts of most plants of this genus have been validated by several studies **[19, 20]**. Phytochemical screening studies proved that, *Cleome* enrichment with a diverse array of beneficial secondary products including essential oils, terpenoids, flavonoïds, phenolics, and alkaloids, supporting use of the genus for culinary and therapeutic purposes**.** This study summaries the chemical constituents, uses, and pharmacological activities of *Cleome* genus **[21, 22]**.

Interest in the phenolic compounds has greatly increased recently because these compounds have been implicated in suppressing the risk of degenerative diseases in humans. The pharmacological properties of plants may be related to their antioxidant capacities and hence there was need to investigate the antioxidant potential using aqueous, hexane and acetone extracts of different parts of *C. gynandra* **[23]**.

However, no scientific literature has been reported on the antioxidant and phytochemical properties of C. gynandra in Benin in spite its paramount ethnobotanical importance in small households. Therefore, this study was undertaken to evaluate quantitatively the compositions of phytochemicals and antioxidant properties of acetone, water and hexane extract of different parts (leaves, stem, seeds) of C. gynandra L. in order to justify its ethno-medicinal importance in Benin. The overall goal was to contribute to the possible domestication of this wild vegetable in order to explore the pharmacognosical and therapeutic potentials which would broaden the food base in the whole Benin and West Africa. Thus, total phenolic, total flavonoïds, tannins, alkaloids in two provenances of spider plant collected in contrasted agro-climatic zones (Sudanese [Northern] and Guinean [Southern] climates) in Benin have been investigated. Specific objectives of the present study were therefore: (i) to evaluate the type and amount of phytochemicals and the changes caused by provenances and part of the plant used; (ii) and to assess the *in vitro* antioxidation properties of the various plant parts and provenances of spider plant.

Material and Methods

Plant Material

Fresh leafed branches, stems and seeds of *C. gynandra* were collected from the Southern (Dogbo) and Northern (Tanguiéta) Benin (*Table 1*). Dogbo is located in the Department Couffo at 80m of altitude

and 03°65'23 "8 North latitude and 07°51'76 "0 East longitude, whereas Tanguiéta is in the plains of the Atacora Department at 234 m of altitude and is situated between 03°11'39 "4 North latitude and 11°75'23 "5 East longitude. The climate is of Guinean type in the Southern and Sudanese in the Northern. The plant was identified at the Department of Plant Biology; University of Abomey-Calavi.

Table 1: Plant Material used, climatic data of provenance origins and extract nature

Climates	Provenances	Plant organ used	Extraction solvent	Nature of extract
Southern-Benin (Guinean climate)	Dogbo	Leaf powder Stem powder Seed powder	Acetone Distilled water Hexane	Leaf extract Stem extract Seed extract
Northern-Benin (Sudanese climate)	Tanguiéta	Leaf powder Stem powder Seed powder	Acetone Distilled water Hexane	Leaf extract Stem extract Seed extract

Preparation of Extracts

The plant samples were harvested in July 2015, properly washed, cleaned and dried safe from light to avoid all modification or deterioration of the chemical constituents at ambient temperature to a constant weight, pulverized to fine powder and stored in airtight bottles which were then kept in the refrigerator at 4°C until needed for the analysis. From the powdered samples, 100 g of each plant part (leaves and stem) or 50 g (seeds) was extracted separately in acetone, distilled water and hexane for 72, 24 and 72h, respectively under mechanical agitation. The extracts were then filtered using Whatman paper filters, and evaporated to dryness under reduced pressure at 50°C using a rotary evaporator. The resulting extracts were reconstituted with acetone to give the required concentrations to be used in the study. Extraction to dosages has been carried out according to the methodology of **[24]** and **[25]** with little modifications.

Chemicals and Reagents Used

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), vanillin, butylated hydroxyl toluene (BHT), rutin, potassium persulphate, sodium nitroprusside ($Na_2[Fe(CN)_5NO]_2H_2O$), sulfanilic acid, glacial acetic acid (CH_3COOH), gallic acid, tannic acid, ferric chloride ($FeCl_2$), ascorbic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), potassium acetate (CH_3CO_2K), phosphate buffer, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA). They were purchased from Merck, Gauteng, South Africa. All the chemicals used in this study were of analytical grade.

Phytochemical Screening

Phytochemical screening was fulfilled on the basis of the experimental methodology proposed by **[26]**, relying on differential precipitation reactions and coloration with slight modifications. *Table 2* summarizes the specific reactions for every class of chemical compounds.

Table 2: Outline on specific reactions of each chemical compound class during the phytochemical screening

Classes of active compounds	Specific reagents and reactions
Alcaloïdes	• Dragendorff (potassium iodobismuthate) : precipitate brown or brown-sallow • Mayer (iodomercurate de potassium) : precipitate yellow
Catechic tannins	• Stiasny reagent : precipitate pink
Gallic tannins	Na acetate Saturation + some drops of FeCl3 1% : blue or black hue
Flavonoïds **flavones**	Shinoda (reaction to cyanidine) : coloration orange, red, violet coloration orange

flavonols **flavonones**	coloration red coloration violet
Anthocyanes	Red Coloration of filtrate increased in acidic solution and blue purplish in alkali solution
Leucoanthocyane	Shinoda (alcool chlorhydrique) : coloration red cherry
Guinonic derivatives	Born-Trager (reaction between quinonic cycles in NH3 solution : coloration pink to red purplish
Saponosides	Determination of moss indice (positive if IM > 100)
Triterpenoids	• Liebermann-Buchard (acetic anhydride - sulphuric acid 50 : 1) : coloration purple to blue or green
Mucilages	Study of the viscosity of infusions and decoctions. Floculation in absolue éthanol
Reducing compounds	Hot Fehling Liqueur : precipitate red-brick

Determination of Total Polyphenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method **[27]**. A 125µL aliquot of each plant organ extract (1mg/ml) was mixed with 625 µL of Folin- Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v) and 500 µL (75mg/mL) of sodium carbonate (Na_2CO_3). The tubes containing the mixtures were vortexed for 2 hours and allowed to stand for 30 minutes at 40°C to develop colour. Absorbance was then read at 760 nm using the Biomate mark spectrophotometer. Results were expressed as mg/g gallic acid equivalent using the equation based on the calibration curve: Y = 0,0303x + 0.6579 with a regression coefficient R^2 equal to 0.9777.

Determination of Total Flavonoïds

The flavonoïd content was determined by the method used by **[28]** and **[29]**. Briefly, 500 µL of 2% $AlCl_3$ was prepared in methanol. This was then added to 500 µL of the extracts. To this mixture, 3mL methanol were added. The blank or control was constituted of 500 µL $AlCl_3$ and 3,5mL methanol. The mixture was allowed to stand for 10 min at room temperature and the absorbance was read at 415 nm. The rutine was used to perform the standardization to different concentrations.. Results were calculated as rutine equivalent (mg/g) using the equation based on the calibration curve: y = 0.0875x + 0.029 with a regression coefficient R^2 equal to 0.998; where x is the absorbance and y is the rutine equivalent (EQ).

Polyphenol, flavonoïd and condensed tannin contents in extracts have been determined by the following formula:

$$T = \frac{(C \times V_r)}{(V_p \times C_p)}$$

where T = Compound content; C = Concentration gotten from the standardization curve; Vr = Reaction volume; Vp = extract volume used; Cp = Concentration of the extract used.

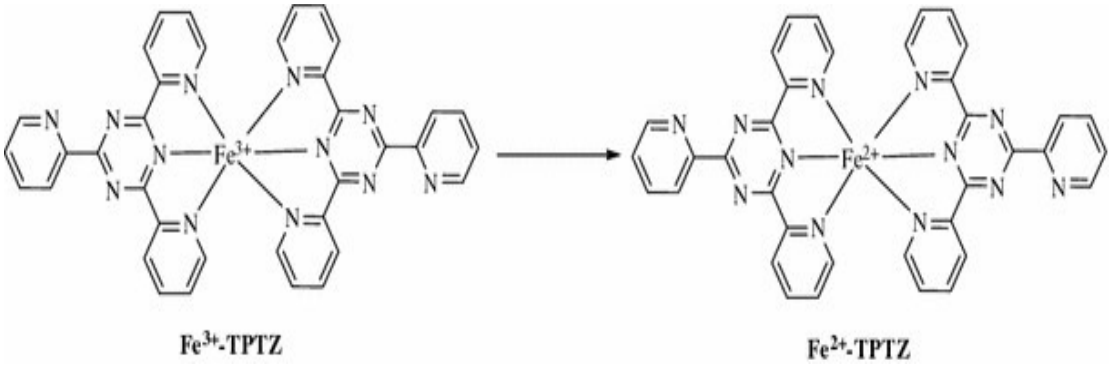
Assessment of the Antioxidant Effect and the Antiradical Activity of Extracts of Cleome Gynandra L. (Capparidaceae)

To assess the antioxidant effect of *C. gynandra* extracts, two methods have been used : the FRAP (Ferric Reducing Antioxidant Power) method, and the 2.2'-diphényl-1-picrylhydrazyl (DPPH) radical utilisation.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the extracts was evaluated according to the methodology of **[30]** with slight modifications. This method measures the reducing power of the antioxidants inside an extract aliquot by their capacity to reduce the ferric tripyridyl-triazine (Fe^{3+}-TPTZ) in ferrous tripyridyl-triazine (Fe^{2+}-TPTZ) in an acidic pH (*Figure 1*).

Figure 1: $[Fe^{3+}$ - (TPTZ) 2$]^{3+}$ - $[Fe^{3+}$ - (TPTZ) 2$]^{2+}$: Reducing reaction in FRAP dosage



For the test, a tampon solution of 300 mM acetic acid/Na acetate pH = 3,6 was prepared. Reagents TPTZ 10mM in HCl 40mM, FeCl3 20mM were used. FRAP was achieved by mixing 3 ml TPTZ, 3 ml FeCl3 and 30 ml tampon solutions. This mixture was kept imperatively in a boiler at 37°C. 100µL extract 1mg/mL was then added to 3000µL FRAP reagent. The mixture was shaken at 37°C for 30 minutes in darkness. The standards: quercetin, luteoline, chrysine, rutine, cafeic acid, chlorogenic acid, ascorbic acid (at suitable dilutions) were applied in same conditions. Absorbances were measured at 593 nm. Ferrous sulphate 0.009, 0.019, 0.039, 0.079, 0.159, 0.319, 0.638 and 1,275mM in ethanol was used to establish standardization curve and equation. For the standardization, a mixture of 3000µL FRAP and 100µL of each stallion solution was used. Blank consisted of 3000µL FRAP reagent mixed with 100µL ethanol. All measures were repeated 3 times.

Determination of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Ability

The effect of acetone extracts from different plant organs vis-à-vis the DPPH free radical scavenging ability was determined using the procedure described by **[31]**. Briefly, a mother solution 1mg/mL of each extract was prepared by dilution 1/10 in methanol. 3ml of DPPH prepared in methanol (Cm = 0,04 mg/mL) were mixed with 1.5ml of different concentrations ranging from 0.0-11.1µg/mL of various *C. gynandra* plant extracts. The mixture was vortexed thoroughly and left in the dark at room temperature for 15 minutes. Absorbance was measured at 517 nm using the spectrophotometer. The scavenging ability of the plant extract on DPPH was calculated using the equation: DPPH scavenging activity (%) C= Clu×D)/(M×Ci)]×100, where C is the antiradical compound concentration in EAA/gMS Extract, Clu the probe concentration, D the dilution coefficient, M the molecular weight of ascorbic acid (vitamin C) (176,1g/mol), and Ci is the mother extract solution concentration in mg/mL.

DPPH solution was prepared from 8mg dissolved in 200 ml methanol. Vitamin C solution was obtained by dissolution of 500mg in 5 ml methanol.

Determination of the Antioxidant Activity (EC50) of the Cleome Gynandra Extracts

For this test, samples were prepared by dissolution in the absolute methanol **[32]**. For each extract, 200 µg/mL mother-solution in methanol was finalized. This solution was then diluted in series to obtain different concentrations consisting of 0.048875, 0.09775, 0.1955, 0.391, 0.781, 1.562, 3.125, 6.25, 12.5 and 25µg/mL. 2 ml extract solution to be tested and 0,8 ml DPPH solution (0,1183 mg/mL) were mixed. After vortexing, the tubes were placed away from light at laboratory temperature for 30 minutes. Absorbance was read at 517 nm with the spectrophotometer (Biomate UV/VIS).

Blanks consisted in mixing 2 mL solution to be tested and 0,8 mL methanol. Positive control was represented by the ascorbic acid (200µg/mL) and was used in the same conditions as the samples to be tested. Extract probes were prepared from 1mg extract diluted in 5 mL MeOH (Cm = 200µg/mL). Positive control was a mixture of 1mg vitamin C and 5 ml MeOH (Cm = 200µg/mL). Negative control

consisted in 0,8mL DPPH solution (0,1183mg/mL) added to 2 mL MeOH. Solution in the DPPH was established as 11,83mg DPPH plus 100mL MeOH (0,1183mg/mL).

The results have been expressed as the average of three repeated measurements ± Standard error. The IC50 value for each extract was determined as the concentration of substratum that causes 50% loss of the DPPH activity (colour), and used to characterize the antioxidant power. ICC values were calculated in % as proposed by **[33]**. Antiradical power (APR) was estimated as inversely proportional to the EC50 (APR = 1/EC50) **[34]**. Antioxidant activity (AA) in % is given by the following formula:

AA(%) = 100 - {[(Abstest - Absblanc) × 100] / Abscontrol}, or : Inhibition(%) = (Abscontrol - Abstest) / Abscontrol X 100.

where AA = Antioxidant activity, Abs = Absorbance at 517 nm.

Statistical Analysis

Statistical analysis of the results was achieved by the software **[35]**. Total polyphenol and flavonoïd contents, and thus antioxidant activities were subjected to two-way analyses of variance at 5%. The factors consisted in the provenance and plant organ of *Cleome gynandra.* Relative importance of each factor to the variation observed was computed as well as their possible interactions with the same software. Moreover, Kruskal-Wallis test implemented in MINITAB 14 was used for the comparison of the provenances and plant organs studied. This test permitted to identify the best provenance as well as the best plant organ with good polyphenol, flavonoïd and antioxidant properties.

Results

Assessment of the Extraction Techniques

Results of the present survey indicate that from 100g powder of leaf branches of *Cleome gynandra* (leaves of the Northern- and Southern-Benin) and 1L, an acetone-based dark green extract containing chlorophyll, flavonoïds, polyphenols and other compounds was obtained. 100g powder stems let getting an aqueous dark brown extract containing chlorophyll alkaloids, flavonoïds and polyphenols. From 50g seeds and ½ litre hexane, we got an green fattening extract rich in oils, carotenes and others compounds.

Phytochemical Test

Phytochemical tests performed on diverse preparations allowed to evidence the presence of many secondary metabolite types in various parts of the plant studied by qualitative characterization (precipitation, coloration by specific reagents, or by under UV light analyses). Results are summarized in *Table 3*.

Table 3: Phytochemistry of *Cleome gynandra*

Phytochemical Compounds	Solvents	Plant organs used *					
		TN	TS	FN	FS	GN	GS
Alcaloïdes	Distilled water	+++	+++				
	Acetone			+++	+++		
	Hexane					+	+
Flavonoïds	Distilled water	+++	+++				
	Acetone			++	++		
	Hexane					-	-
Tannins	Distilled water	-	-				
	Acetone			++	++		
	Hexane					-	-

Phytochemical Compounds	Solvents	Plant organs used *					
		TN	TS	FN	FS	GN	GS
Quinoic derivatives	Distilled water	+++	+++				
	Acetone			+	+		
	Hexane					-	-
Reducing compounds	Distilled water	++	++				
	Acetone			-	-		
	Hexane					-	-
Mucilages	Distilled water	++	++				
	Acetone			-	-		
	Hexane					-	-
Leuco-Anthocyanes	Distilled water	±	±				
	Acetone			-	-		
	Hexane					+++	+++
Anthocyanes	Distilled water	+	+				
	Acetone			+++	+++		
	Hexane					+	+
Saponines	Distilled water	++	++				
	Acetone			+	+		
	Hexane					±	±

(*) – TN, TS, FN, FS, GN and GS: Stem, Leaves and Seeds collected from provenances originated in contrasted agro-ecozones (Northern and Southern) in Benin.

Estimation of Total Polyphenols

Extracts were quantitatively analyzed by spectrophotometer for their contents in total polyphenols using the method of Folin-Ciocalteu. Results are expressed in mg-equivalents of gallic acid (EAGmg) reported to the dry matter in accordance with the linear regression equations of the standardization curve.

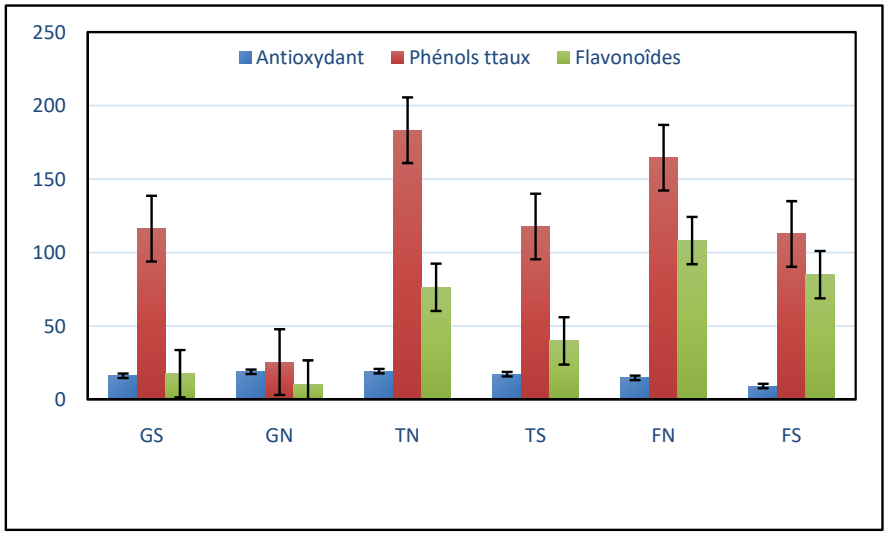
According to the results, we note that all extracts contain many phenolic compounds at very variable concentrations. Aqueous extracts showed the most elevated concentration of total phenol (183.307±2.71mg EAG/g MS) in stems from Northern provenance compared to those from the Southern Benin (117.762±4.2 4mg EAG/g MS). Similarly, leaf acetone extracts showed the same tendency (164.597±4.501mgEAG/gMS in Northern; 112.653± 5.597mgEAG/gMS in Southern Benin). Higher total phenol contents in hexane based seed extracts were observed in Southern than Northern provenance (116.277 ±5.209mg EAG/g MS and 25.477±0.785mg EAG/g MS, respectively) (*Figure 2*).

Dosage of the Flavonoïds

Results showed that all extracts contain a lot of flavonoïds at very variable concentrations too. Acetone-based extracts presented the highest concentration of flavonoïds (108.228±0.069µgEQ/mgMS, and 84.983±0.00µg EQ/mg MS in stems of Northern and Southern provenance, respectively). Stem aqueous extracts showed 76.388±0.000µg EQ/mg MS and 39.908±0.045µg EQ/mg MS for Northern and Southern provenances respectively. Hexane-based extracts contained lower amounts of flavonoïds in the seeds both in Northern and Southern accessions (17.554±0.046µg EQ/mg MS in Southern against 10.537±0.069µgEQ/mgMS in Northern) (*Figure 2*).

Differences were observed in antioxidant power of extracts with relevance to the plant organ and the agro-ecological origin considered. Stem aqueous extract from the Northern Benin demonstrated the highest oxidative inhibition percentage (19.33%) compared to the Southern (17.25%). Leaf acetone-based extracts of the North inhibited (14.69%) more those of the South (9.15%). Seed hexane-based extracts were more antioxidant in the North (18.85%) than in the Southern (16.17%) (*Figure 2*).

Figure 2: Compositions in total phenols, flavonoïds and assessment of their respective antioxidant activities



Diversity in Total Phenol and Flavonoïd Contents According to Provenances, Plant Organs and their Interaction

Table 4 indicates the results out of the analysis of variance for the total phenol and flavonoïd contents in different plant organs and provenances of *Cleome gynandra* collected in Benin. Apart from total phenol and flavonoïd contents where provenance effect was not significant on the results observed, plant organ as well as Provenance × Plant Organ interaction effects were highly significant on the expression of total phenol and flavonoïd contents in *Cleome gynandra* investigated ($p<0.05$) (*Figures 3, 4*).

Figure 3: Mean comparisons for polyphenol (A) and flavonoïd (B) contents by provenance and plant organ in *Cleome gynandra* from Benin

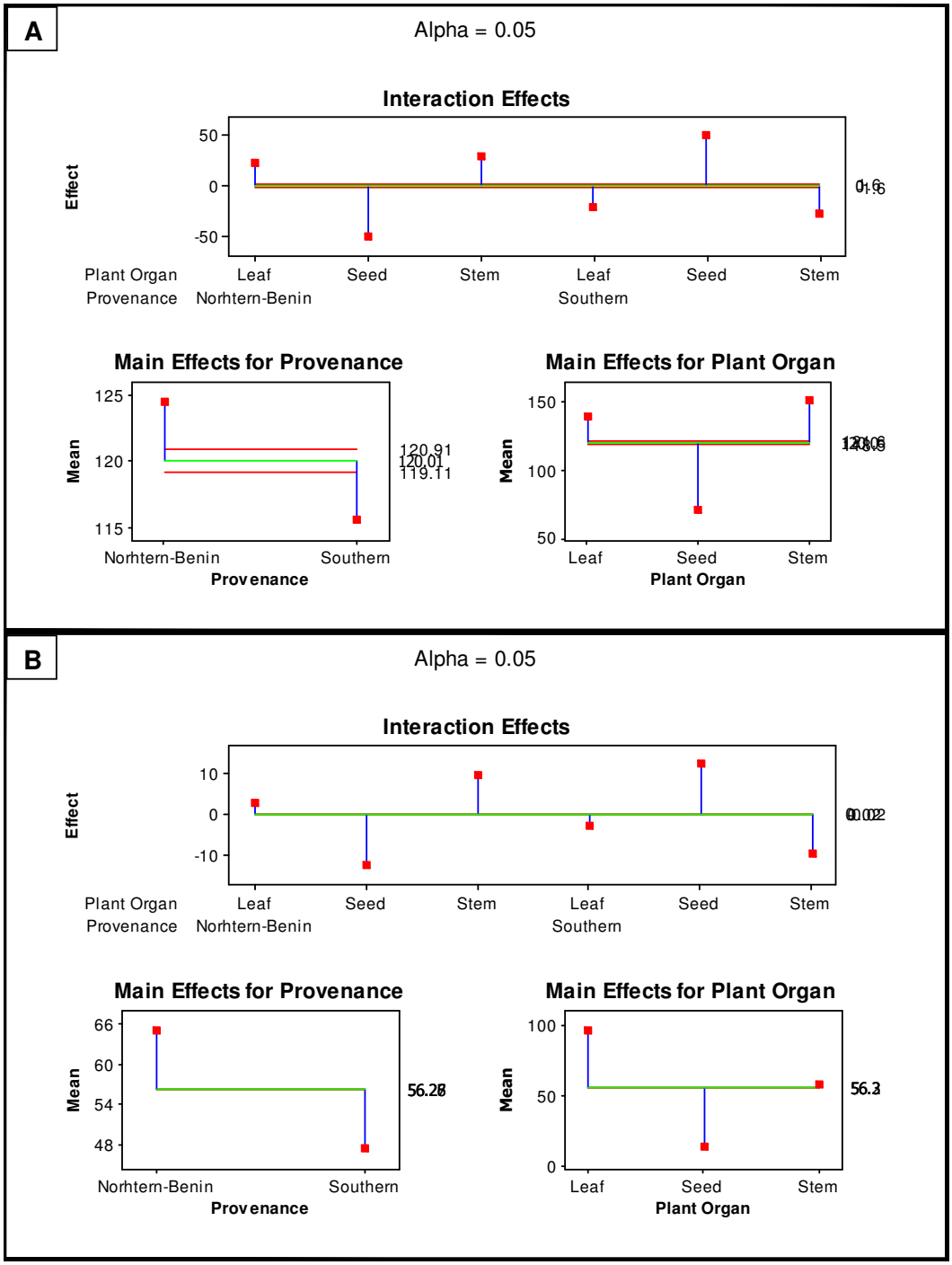


Figure 4: Interaction plots (data means) for polyphenol contents (C); and for flavonoïd contents (D) in *Cleome gynandra* from Benin

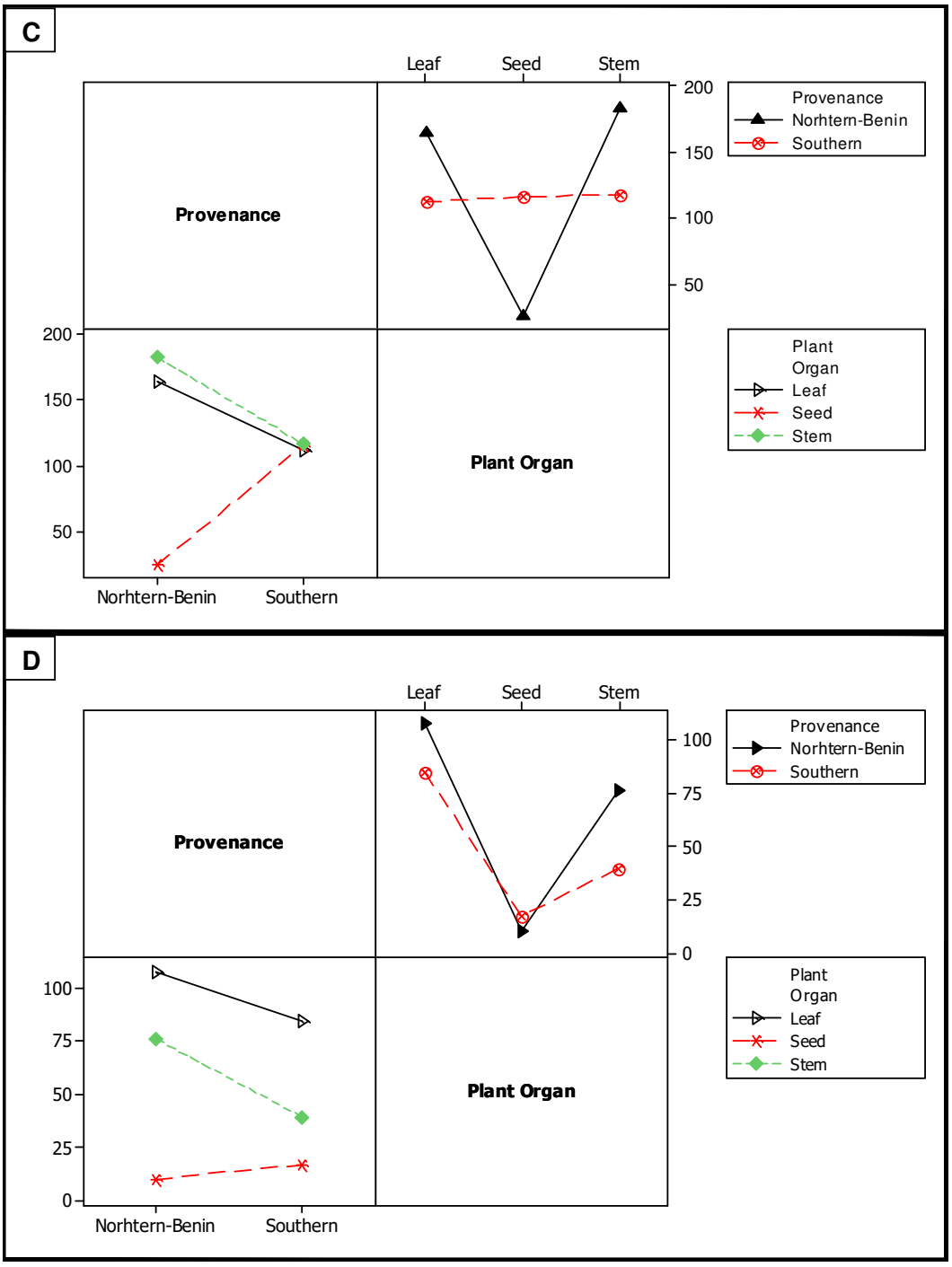


Table 4: Two-way ANOVA for total phenol and flavonoïd contents in different plant organs and provenances of *Cleome gynandra* collected in Benin

Parameter	Source of variation	Degee of freedom	Sum of square	Mean square	F Ratio	Prob> F*
Total Phenol Content	Total variance	35	90234	-	-	-
	Provenance effect (1)	1	712	712	0.03	0.875
	Plant Organ effect (2)	2	44308	22154	3174.31	0.000
	Interaction (1×2)	2	45004	22502	3224.23	0.000
	Residuals	30	209	7	-	-
Flavonoïd Content	Total variance	35	46721.7	-	-	-
	Provenance effect (1)	1	2778.2	2778.2	1.86	0.306
	Plant Organ effect (2)	2	40960.5	20480.3	22613641.55	0.000
	Interaction (1×2)	2	2983.0	1491.5	1646858.24	0.000
	Residuals	30	0.0	0.0	-	-

* (Prob<0.05) : Significant factor effect according to F-test at 5% threshold.

Applying Kruskal-Wallis non-parametric test to detect provenance and plant organ effect on phytochemical and thus antioxidant properties in *Cleome gynandra*, the sample median values recorded for the three plant organs used with regard to total phenol content were 68.32, 151.83 and 139.60 mg EAG/g MS in seeds, stems and leaves, respectively. The z value for leaves is 0.44, the smallest absolute value of z. This dimension shows that the mean rank for this organ differs least from the overall mean. The mean rank of this plant organ (19.6) was revealed nearly equal to the overall mean rank (18.5), and the value of z is positive (z = 0.44). For flavonoïd content, the z value for stems is rather the smallest absolute value (z=0.00), and their mean rank (18.5) is equal to the overall mean value. The mean rank for leaves was higher (30.5), with a positive z value (z = 4.83) (*Table 5*).

The test statistics (H = 14.17 or 14.18) for total phenol content had a p-value 0.001 when it is adjusted or not, indicating that the null hypothesis can be rejected at levels superior to 0.002 in favour of the alternative hypothesis of the existence of at least a difference among plant organs. The probability being inferior to 0.05, the differences between the plant organs are therefore significant. For flavonoïd content, the test statistics (H = 31.14 or 31.26) had a p-value 0.000 whenever adjusted or not, showing high significant differences between all plant organs analyzed (p<0.05). *Cleome gynandra* leaves contained higher amounts of flavonoïds (96.57 µgEQ/mgMS as mean value) (*Table 5*).

Table 5: Kruskal-Wallis non-parametric test of total phenol and flavonoïd contents comparisons in three plant organs of *Cleome gynandra* accessions collected in different agro-ecozones in Benin

Parameter	Factor		N*	Median	Average rank	z
Total Phenol Content	Plant Organ	Seed	12	68.32	9.9	-3.46
		Stem	12	151.83	26.0	3.02
		Leaf	12	139.60	19.6	0.44
		Overall	36	-	18.5	-
	Provenance	Northern-Benin	18	164.60	21.5	1.71
		Southern-Benin	18	116?30	15.5	-1.71
		Overall	36	-	18.5	-
Flavonoïd Content	Plant Organ	Seed	12	14.06	6.5	-4.83
		Stem	12	58.17	18.5	0.00
		Leaf	12	96.57	30.5	4.83
		Overall	36	-	18.5	-
	Provenance	Northern-Benin	18	76.39	19.5	0.57
		Southern-Benin	18	39.91	17.5	-0.57
		Overall	36	-	18.5	-

(*) : Number of observations.

The calculation of the sample medians for both provenances investigated delivered 164.60 and 116.30 mg EAG/g MS total phenolic content for provenance 'Northern' and 'Southern', respectively. The z value for 'Southern' is -1.71, the smallest, but equal in absolute value to the z for accession 'Northern' (*Table 5*). This observation is showing that the mean rank between provenances differs highly from each other conversely to the observations with simple two-way ANOVA. For flavonoïd content, similar observations were made with provenance 'Southern' presenting the highest z of +0.57 (*Table 5*).

For the total phenol content, the Kruskal-Wallis test statistics (H = 2.92) had a p-value of 0.088 or 0.087 whenever adjusted or not, showing that the null hypothesis can be rejected at levels superior to 0.088 or 0.087 in favour of the alternative hypothesis of the existence of at least a difference among provenances. The probability is distinctly higher than 0.05 (*Table 5*). Thus, differences between provenances are indeed not significant. Similar results were noted for flavonoïd content between provenances with H = 0.32 and 0.33 when adjusted and p-values equal to 0.569 and 0.566, respectively. These results are here also contrasting with the above mentioned ones in ANOVA (*Table 4*).

Discussions

Phytochemical screening carried out on diverse *Cleome gynandra* plant organs revealed their richness in many secondary metabolites that are interesting and useful for the traditional medicine. Classes of those metabolites identified encompass alkaloids, flavonoïds, tannins, quionine derivatives, reducing compounds, mucilages and leuco-anthocyanes. These metabolites were encountered in *Cleome gynandra* collected in two different climatic zones in Benin, the Northern and the Southern (*Table 1*). This result is congruent with the reports of **[25, 36, 37]**.

According to the results of the seed phytochemical screening indicated that they are very rich in leuco-anthocyanes. Tannins and saponins are inside low. Apart from differences due to plant organ considered, environment played a paramount role in the phytochemical composition of *Cleome gynandra* suggesting that material from the Sudanese Climate (in the Northern) are likewise more rich in almost chemical compounds. This fact is contrasting with the recent findings reported by **[9]** signalling that accessions from Sudanese climate (Northern-Benin) germinate rather slower than those from Guinean zones with higher germination capacity and speed.

The results of dosage revealed that the stem aqueous extract of the Sudanese climate provenance (Northern) presented the strongest concentration of total phenol (183.307±2.71mgEAD/gMS) followed-up by acetone-based leaf extract (164.597±501 mgEAD/gMS) and hexane-based seed extracts (116.277±5.209 mgEAD/gMS) under the same climate, respectively (*Figure2*). In relation to the leaf contents in flavonoïds, probes from the same climate (Northern) presented 108.228 ±0.069µgEAD/gMS, whereas those of the Southern (Guinean climate) contained in average 84.983±0.000µgEQ/mgMS followed by the stem extracts of the Northern (76.385±0.000µgEQ/mgMS) and those of the Southern (39.908±0.045µgEQ/mgMS) (*Figures 2-4*). Results showed a decreasing tendency from Northern to the Southern in relation to the seed contents (17.554±0.046µgEQ/mgMS in Southern and 10.557±0.069µgEQ/mgMS in Northern). These results show that all parts of the plant are useful and could bring us to priories the species of the north that those of the south.

In general, polyphenol contents in different parts of the plant were significantly high. They could be responsible for the strong antioxidant activity of the *C. gynandra* extracts. The high concentrations in flavonoïds, phenols, and biological substances that inhibit or heal a lot of pathologies may deal with mechanisms involved in defences against severe diseases. Thus, they should be included in fact within classes of antimicrobial and antioxidant compounds that encourage the cytotoxicity in microbes **[38, 39]**. These compounds are also very essential because of their capacity to recover their hydroxyle groups, neutralize the free radicals and decompose the peroxides **[40]**. For example, the

flavonoïds and phenols act as good traps of lethal radicals preventing lesions thus cellular oxidations. They are also known to have various pharmacological and biological effects, anti-inflammatory properties, immuno-modulator and anti-allergic activities that reduce the cardiovascular mortality risk **[41]** mentioned by **[23]**. These are important elements that could be taken into account to validate the traditional uses of the species *Cleome gynandra*. For instance, Flavonoïds and phenols acts as good scavengers of lethal radicals thus preventing oxidative cell damage. They are also known to have various pharmacological and biological activities such as anti-inflammatory, immunomodulatory and anti-allergic properties which reduce the risk of cardiovascular mortality **[41]**. The amount of proanthocyanidin in this study was observed to be the highest among the other polyphenols. Proanthocyanidins are known to possess strong antioxidant property. It has been shown that proanthocyanidins reduces the risk of cardiovascular disease, cancer and improve blood circulation by strengthening the capillaries **[17, 42]**. Thus, the strong antioxidant activity exhibited by the extracts from C. gynandra could be ascribed to the high polyphenols, micro and macro mineral elements present in the different plant parts. This partially validates the utilization of this plant in folklore remedies for the treatment of diseases. Tannin content was relatively low and similar in all parts of the plant. Nevertheless, this low concentration of tannin can still contribute significantly to the body. They play a major role in the treatment of inflamed tissues as well as prevention of cancer **[43]**. Also, the alkaloid and saponin contents were low in this species. These compounds are known to have ethno-pharmacological uses for instance; alkaloids act as agents which possess analgesic and anti-pasmodic properties while saponin acts as antifungal agents. Inspite of its medicinal characteristics, saponins are noxious to humans as they cause heamolysis of blood and irritation of mucous membranes **[18]**. The low concentration of saponin and alkaloid observed would suggest low toxicity in the plant.

[44] stated that location influence the mineral and trace element compositions of rice, wheat, oats and barley and these are mainly attributed to the altered soil conditions and that the nature and chemical composition of the soil are also involved in location differences in mineral elements. **[45]** stated that variations in the chemical compositions of leafy vegetables, including quantity of compounds that are useful and detrimental to humans are influenced by environmental conditions and the age of plants at harvest. Phytochemicals present in most vegetables crops are very susceptible to changes in environmental conditions. Phenolic contents are affected by biotic stresses (insect attack and pathogen infection) and abiotic stresses (light, temperature, nutrient supplies, water availability, growing conditions and UV radiation) besides storage conditions, post-harvest treatments and the estimation methods **[44, 46]**.

These conditions, besides the biosynthesis of phenolic antioxidant compounds, affect the final concentration of polyphenols in plant tissues. Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway. Phenylalanine ammonialyase (PAL), the key enzyme catalyzing the biosynthesis of phenolics from the aromatic amino acid phenylalanine, has been found to be responsive to biotic and abiotic stresses **[47]**.

Phenolics are distributed differently depending on the crop and on the plant part evaluated. External and internal leaves of different *B. oleracea* crops like tronchuda cabbage **[48]** savoy cabbage **[49]** were found to be different in terms of total phenolic content. Quercetin, kaempferol and phenolic acids derivatives from the external and internal leaves, seeds and sprouts leaves of tronchuda cabbage have been reported by several researchers **[48, 50]** and the different composition seems to be determinant for the antioxidant activity displayed by each.

Biosynthesis and concentration of phenolic compounds in plants depends on genetic and environmental factors. Several studies have demonstrated that there is a substantial and significant variation for the antioxidant phytochemicals in vegetables.

For instance, *Brassica* species, exhibit variation both within and among species, and even among crops of the same species; thus, the potential health benefits provided by cruciferous crops will depend firstly on the genotype. The phenolic compound composition may differ between cultivars, as

well as among parts within the individual plant as shown in several crops like turnip greens and turnip tops **[51]** and tronchuda cabbage according to **[52]**.

Secondary metabolites are also known to vary in amount and content depending on the age of the plant **[53]**. Although it has been reported that there exists a large variability in the levels of phenolic compounds at various stages of maturation **[54]** of some leafy vegetables, the changes that occur in the content of these chemicals at different growth stages of traditional leafy vegetables found in Sub-Saharan Africa is poorly understood. Even so one needs to know the best time to harvest. Nutritional quality of vegetables is dependent on factors like variety, degree of maturity and the weather conditions during growth **[55, 56]**. It can also be influenced by post harvest handling and storage **[57-59]**. There is higher amount of vitamin C present in ripe mature vegetables, whose content increases progressively in advance stages of maturity according to **[60]**.

Higher amounts of phenolic compounds compared to flavonoïds seem logical since flavonoïds represent the major parts of polyphenols and constitute therefore a part of the total phenols. It can also be explained by an increase of the phenolic metabolism of the plant; in addition to the existence of a link with climatic conditions, as elevated temperatures, day-length, the nature of soil and the growth season **[61, 62]**. It is especially true than the dosage of the leaf total phenols based on acetone extraction indicated different results between locations of provenance (leaf of the North: 164.597±4.501; leaf of the South: 112.653±5.597mgEAD/gMS).

The potential of the *C. gynandra* extracts in reducing Fe^{3+} into Fe^{2+} showed the reducing capacity of the plant in the pharmacological point of view. The capacity of aqueous reduction of the Northern stem followed by seed extracts in n-hexane in this species was significantly higher than antioxidant ability of the other extracts (*Figure 2*). The different parts of the plant presented a higher capacity to ferric reduction compared to the norms. At the lowest concentration, the stem extract showed the strongest ferric reduction capacity, whereas the seed extracts presented a different tendency by more elevated concentration. Indeed, an increase of the absorbance is indicating a drop of the reducing capacity of the plant. It implies that the dose of lowest concentration of plant extract is very active even in accordance with enzyme activity laws. The reduction of the extract probes followed the following tendency: stem > seed > leaf > rutine > vitamin C. The reducing potential of extracts decreased with increase of the concentration in active compounds. Extracts from different plant organs presented higher reducing capacity at lower concentration. These results corroborate the reports of **[23]** demonstrating that extract reducing capacity follows this tendency: stems > fruits > leaves. The lower concentration of pure extract seems very powerful in reducing the ferricyanure into its ferrous form. Besides, plant extract activities were significantly more elevated than those of known norms. Their reducing potential observed results from the presence of the polyphenolic absorbent compounds inside **[23, 63, 64]**. Our observation is fully in agreement with the results of other authors that showed close relationships between the reducing potential of the plant extracts and the quantity of constituents represented by phenolic compounds **[65]**. Moreover, the antioxidant effect of an extract can also differ according to the quality of polyphenols present such as flavonoïds which show remarkable antioxidant activities **[66]**. Genistein and daidzein appear in the phenolic composition of methanol-based extract of *Retamasphaerocarpa*, and showed high antioxidant activity due to the direct chemical effect caused mainly by their phenolic structures as demonstrated by **[67]**. The mechanism of the reaction between the antioxidant and the DPPH depends upon the structural conformation of the antioxidant **[68]**. Some compounds react very quickly with the DPPH while reducing a number of DPPH radicals equal to that of the hydroxyle groups of the antioxidant **[69]**. The spatial configuration and the number of groups -OH in the flavonoïdic structures can influence the different antioxidant mechanisms involved **[69]**.

Since several researches showed that the antioxidant effect of natural resources is bound to the presence of phenolic compounds **[70-73]**, the aqueous extract that demonstrated the highest content in polyphenols and the best antioxidant activity in this test, let conclude that the strong antioxidant activity of the aqueous hexane-based extracts in *Cleome gynandra* is due to their countenance in phenolic compounds.

The scavenging activity of the different parts of C. gynandra extracts against free radicals was plant organ dependent (*Figure 3*). Vitamin C activity was significantly higher than rutin and the extracts. The activities of the extracts from the stem, leaf and seeds against free radicals were significantly ($P < 0.05$) different from each other as indicated by their phenolic and flavonoïd content variability (*Tables 4, 5*). Antioxidant activity assay is based on the inhibition of the absorbance of the radical cation. The scavenging activity of the various plant extracts against ABTS radical was found to be higher in the leaf than the stem and seeds. Although all plant parts exhibited their highest inhibition at a concentration of 0.50mg/ml. This could imply that the scavenging activity by the plant extracts is dose responsive and could be helpful in treating radically related diseases especially at higher concentrations **[74, 75]**. The trend of the inhibition of DPPH radical by the extract was concentration dependent. With respect to the IC50 values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend: vitamin C < rutin < acetone leaf < acetone seed < acetone stem (*Tables 2, 3*). It is interesting to note that the lower the IC50 value, the higher the scavenging activity of the plant extract. The results of the DPPH also showed that the standard drugs were not significantly different ($p<0.05$) from each other. On the other hand, the fruit extract showed the least scavenging activity amongst the plant extracts. The DPPH assay is based on the principle that the antioxidant compounds will scavenge the DPPH radicals in order to form constant reduced DPPH molecules. When these molecules are formed, the absorbance decreases and the DPPH solution decolourises from purple to yellow. The degree of discolouration is an indication that the plant extract has the potential to scavenge free radicals as a result of its ability of hydrogen donation. More yellowish colour of DPPH is an indicator of stronger antioxidant activity of the extracts **[76]**. The DPPH scavenging activity of the various plant extracts and standards were dose responsive. It was observed that vitamin C had the highest activity, followed by rutin, acetone stem, leaf and fruit respectively. Although the DPPH radical scavenging ability of the extracts was significantly lower than that of rutin and vitamin C. The result obtained from this study is congruent with the findings of **[77]** who reported the scavenging activity of Leonotis leonurus to be lower than that of the standard drugs. It was evident that the plant extracts showed proton-donating potential which could serve as free radical inhibitors.

Spider plant is reported to deliver health benefits in addition to fulfilling physiological nutritional needs **[78-80]**. Their consumption is associated with protection against major diseases including cancer and cardiovascular diseases **[80-82]**. The protective action of the vegetables has been attributed to the presence of antioxidants **[83, 84]**. Research has shown that the majority of the antioxidant and antimicrobial activities may arise from secondary metabolites rather than just from vitamins C and E, and β-carotene alone **[85]**. Moreover, a positive correlation between total phenolics and antioxidant activity in this vegetables and fruits has been reported **[57, 86, 87]**. **[88]** stated that quercetin is one of the strongest antioxidants among flavonoïds. It is reported that they chelate metals, scavenge oxygen free radical and prevent oxidation of low density lipoprotein in *vitro* studies **[89, 90]**. Earlier studies have established the abundance of antioxidants in spider plant leaves **[87, 91]** and that there was a general trend towards increased antioxidant activity with increased total phenolics content. These beneficial effects of phenolics make it necessary to understand the circumstances under which they are synthesized and accumulated in these plants. Spider plant accumulates high levels of anti-nutritional factors such as nitrates in addition to their high nutritional value. This has long been a concern to human nutrition and health **[92]**. Research studies **[93, 94]** have shown that availability of plant nutrients can be an important factor in determining the activity of secondary metabolism within plants.

Surveys have shown that spider plant is among the traditional leafy vegetables whose consumption is on the increase in the Tropics and Subtropics, particularly in Kenya **[95]**. However, due to lack of improved varieties and lack of fertilizer use has led to low yields of the crop **[96, 97]**. Since the vegetable is a C4 plant it is expected to do very well in both the tropics and sub-tropics **[98]**. But

this has not been achieved due to poor agronomic practices like lack of research information regarding various varieties together with their optimum handling and nutrient handling during cultivation to optimize phytonutrient quality. Presently, there are many genotypes of spider plant at offer **[99]**. However, majority of these varieties are yet to be characterized in terms of their nutritional and phytochemical quality in relation to nitrogen application or different stages of harvest. The organoleptic properties like taste, appearance and texture differ widely among varieties and across stages of development due to accumulation of phenolics and fibre which influence them **[100]**.

The plant also was shown to have anti-tick properties **[101]**. *Cleome gynandra* L. has traditionally been used for the treatment of rheumatic and other inflammatory conditions as reported by **[102]**. In recent years, phenolic compounds, which are in abundance in spider plants, have been intensively investigated because of their potential health-promoting effects **[103, 104]**. They have been reported to possess many useful properties for human health, including anti-inflammatory, enzyme inhibition, antimicrobial, antiallergic, vascular and cytotoxic antitumor activity, but the most important action of phenolics is their antioxidant activity **[105, 106]**. Furthermore, phenolic compounds possess other properties such as hydrogen peroxide production in the presence of certain metals, the ability to scavenge electrophiles and inhibit nitrosation reactions and chelate metals and, therefore, they act by blocking the initiation of several human diseases **[107-109]**. The antioxidant activity of phenolic compounds is related with its chemical structure that confers them redox properties. They can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides. Reactive oxygen species, derived from oxidation processes, are an important part of the defence mechanisms against infection, but excessive generation of free oxygen radicals may damage the tissue.

Of paramount importance are their antioxidant activity and their wide range of pharmacologic properties of the spider plant **[81]**. They have been associated with anticarcinogenic and antiarteriosclerotic properties and they have the ability to fight dental carries and diarrhoea **[84, 110, 111]**. The polyphenols are also considered to have potential for the management of HIV/AIDS. For instance, caffeic acid derivatives (such as dicaffeoylquinic and dicaffeoylytartaric acids) have been shown to selectively inhibit Human Immunodeficiency Virus type1 (HIV-1) integrase **[112]**. In addition, *in vitro* studies have shown that quercetin prevents oxidation of low density lipoprotein **[90]**, a process which is thought to be an intermediate step in the formation of ateriosclerotic plaques **[88]**. It is possible that the preference for spider plant vegetables shown by some communities arises not only from desire to enrich their diet, but also from their ability to prevent and cure diseases.

Conclusion

This study revealed significant differences between plant organs as well as agro-ecological origins of *Cleome gynandra* used indicating diversity in the substantial countenance in phenolic and flavonoïdic compounds of high medicinal value. These phytochemical constituents are known to be responsible for the strong antioxidant activity exposed by this species. The antioxidant potential of the extracts revealed that all parts of this plant possess the capacity to recover free radicals. Provenance from the Northern-Benin (under Sudanese climate) seems to benefit an advantage over Southern provenance (under more humid Guinean climate). Among the diverse extracts tested, the aqueous seems to give better results and could therefore confirm its therapeutic use in the traditional medicine since it may be easily available to the small and poorest households. Significant diversity with relevance to provenances and plant organs considered shows the necessity for further investigations to identify more material aiming at improvement programmes.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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