



Antioxidant Activity, Total Phenolic and Flavonoid Contents of *Sesbania pachycarpa* (Fabaceae) Leaves' Extract

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ABSTRACT

The aim of this present work was to screen the phytochemicals compounds and, to determine the total flavonoid and total phenolic contents as well as an antioxidant potential of an aqueous extract of *Sesbania pachycarpa* leaves. During this process, the quantities of total phenolic content of the extract were measured using the Folin-Ciocalteu reagent, whereas aluminum chloride colorimetric method was analyzed for total flavonoid determination. The antioxidant activity of leaf extract was determined by the 1,1-Diphenyl-2-Picrylhydrazyl radical scavenging (DPPH) activity test, whereas the Ferric Reducing Antioxidant Power (FRAP) activity was determined through spectrophotometrically analysis. The results showed that the aqueous extract exhibited the altered DPPH free radical scavenging activity ($IC_{50} = 0.921$ mg/mL) and FRAP activity tests ($IC_{50} = 0.173$ mg/mL) compared to that of the positive controls ascorbic acid ($IC_{50} = 1.099$ and 0.33 mg/mL respectively). The amounts of total phenolic and total flavonoid contents of the leaf extract were 141.56 mg Ga E/ 100 grams and 67.03 mg RE/ 100 grams of extract, respectively. Results of the phytochemical components had screening showed the absence of tannins, glycoside, triterpenes/steroids and saponins. Our findings suggest the possibility of using the leaves as a novel source of natural antioxidant agent for the pharmacology.

Keywords: *Sesbania pachycarpa*, Ascorbic acid, Gallic acid, Anti-oxidant, IC_{50}

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INTRODUCTION

Natural substances from plants have antioxidant interests because of their redox property which allows them to act by reducing oxidants¹. Among these compounds, we find to a large extent the secondary metabolites which have been particularly illustrated in therapeutics and which have the advantage of being of a great diversity of chemical structures².

In recent years, there has been a renewed interest in the study of the antioxidant activity of medicinal plants because of the role it plays in the prevention of chronic diseases associated with aging such as

pathologies of the heart, cancer, diabetes, arteriosclerosis, and Alzheimer's disease by fighting oxidative stress^{3,4}. Indeed, the exaggerated overproduction of oxidants beyond the antioxidant capacities of the biological systems of the organism give rise to pathological states such as alterations in proteins, DNA damage, or damage to the integrity of the cell membrane by the induction of lipid peroxidation^{5,6}. This process of oxidative stress can be caused by the nutritional deficit in antioxidants like vitamin E, vitamin C, carotenoids, polyphenols, and also by pro-oxidative factors such as tobacco, alcohol, metals, as well than a decrease in

antioxidants following an alteration of defense mechanisms⁷.

The action of free radicals can however be countered by antioxidants from plants which prove useful and without side effects in comparison to synthetic anti-antioxidant chemicals such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), propylated gallate (PG) and tetra-butylhydroquinone (TBHQ) which are not devoid of toxicity^{8,9}. It was with a view to replenishing antioxidants from plants used in traditional medicine that the choice fell on the leaves of *S. pachycarpa*.

S. pachycarpa belongs to the Fabaceae family. *S. pachycarpa* is an upright, sublimb plant, up to 2 to 4 m tall. It has alternate leaves composed paripinnate with very numerous leaflets. It has a rod which is cylindrical and solid. The leaves are compound and alternate. The flowers are assembled in small clusters of 3 to 6, in the axils of the leaves. This species is common in West and Central Africa¹⁰. This plant is used in traditional medicine in Burkina Faso to treat schistosomiasis¹¹. Described by Nacoulma¹², the leaves are used in external use to treat wounds, varicose ulcers, ear infections and in internal, malaria, helminthiasis (nematodes), febrile body aches, gastritis. Phytochemical screening, showed coumarins, flavonoids, tannins, phenolic compounds, alkaloids, saponosides, and triterpens and/orsteroids¹³. The present study aimed to screen the phytochemicals compounds and, to determine the total flavonoid and total phenolic contents as well as an antioxidant potential of an aqueous extract of *Sesbania pachycarpa* leaves.

MATERIALS AND METHODS

Collection of Plant Material

In September 2019, the fresh leaves of *S. pachycarpa* were collected from Adamawa Region (Ngaoundéré, Cameroon). The plant was identified by Professor Pierre-Marie Mapongmetsem, Professor of plant taxonomist in the Biological Sciences, Faculty of Science, University of Ngaoundéré, Cameroon. The voucher specimen was deposited under the reference number at the Cameroon National Herbarium at Yaoundé, Cameroon.

Aqueous Extract Preparations of Plant Material

The fresh leaves of *S. pachycarpa* were washed and dried in darkness for six days at the laboratory temperature (25 °C) and further ground in a mortar to make the powder, after which the ground leaves were extracted with distilled water with the proportion of 1: 10 (w/v). In brief, one hundred grams of plant material powder was put into conical flask and one thousand milliliter of distilled water was added. The mixture was placed in water bath at 45 °C for 20 minutes. The extract obtained was combined, filtered, and concentrated by evaporation at 45 °C under reduced pressure in a rotary evaporator (Heildolph®). The dried extract was kept at room temperature in sterile sample tube for the further experiment.

Chemicals

Gallic acid, ascorbic acid, Folin-Ciocalteu reagent, aluminium chloride, ferric chloride (FeCl₃), 1,1-diphenyl-2-picrylhydrazyl (DPPH), rutin, sodium acetate (Na₂CO₃), potassium ferricyanide (K₃Fe(CN)₆), and trichloroacetic acid were purchased from Sigma-Aldrich (St Louis, United States of America). All chemicals and reagents were of analytical grade.

Qualitative Phytochemical

Preliminary qualitative phytochemical screening to check the presence or absence of bioactive compounds was carried out according to standard methods^{14,15,16}. The tests were based on the visual observation of color changes or formation of a precipitate after the addition of specific reagents.

Quantitative Phytochemicals Test

Determination of total flavonoid content

To quantify total flavonoid content was determined according to Quettier-Deleu et al.¹⁷. Flavonoid form yellowish complexes in the presence of aluminum chloride, thanks to the free hydroxyl groups. The crude sample was prepared by liquefying 10 mg of the extract in 10 mL of the solvent to yield a concentration of 1 mg/mL. The sample contained 1 mL of methanolic solution of the extract in the concentration of 1mg/mL, 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It remained at ambient temperature in the dark for 30 min; the absorbance of the reaction mixture was measured at $\lambda_{\max} = 510$

nm using the UV 2100 Spectrophotometer (UNICO®). The same procedure was repeated for the rutin and the calibration line was construed. Concentration of flavonoid in aqueous extract was expressed in terms of rutin equivalent (mg ER/ 100 g of extract).

Determination of total phenolic content

In order to estimate the quantity of total phenolic contained in the aqueous extract of *S. pachycarpa*, the methods of Chang et al.¹⁸ with slight modifications was used. This method depends on the reduction of Folin's reagent by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm¹⁹. A dilute extract of methanol solution of extract (0.5 ml of 1 mg/ mL) were introduced into test tubes, and was mixed with Folin Cio calteu reagent (2.5 mL, 1: 10 diluted with distilled water) and aqueous sodium carbonate solution (2.5 mL, 1 M). Gallic acid (positive control) at various concentrations of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL were concomitantly prepared in methanol. The mixture was shaken and incubated at room temperature in the dark for 30 minutes and then, total phenolic were determined by UV-2100 Spectrophotometer (UNICO®) at 765 nm against a blank with distilled water. The sample was carried out in triplicate and the mean value of absorbance was obtained. Total phenolic contents were expressed as mg of gallic acid equivalent / 100g of extract (mg GaE/ 100g Ex.).

Antioxidant Activities

Antioxidant capacity of the aqueous extract was examined using 1,1-Diphenyl-2-Picrylhydrazyl radical scavenging activity test and ferric reducing antioxidant power activity, because evaluation of antioxidant properties of plants cannot be carried out accurately by single universal method.

Ferric reducing antioxidant power (FRAP) activity

The ability of the plant extract and reference substance to ferric reducing antioxidant power (FRAP) activity were estimated using the method described by Oyaizu²⁰. Briefly, 1 mL of plant extract (1 mg/mL) was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of aqueous potassium ferricyanide solution (1 %, w/v). After 30 min incubation at 50 °C, 2.5 mL of trichloroacetic acid

solution (10 %) was added and the mixture was centrifuged at 3000 rpm for 10 min. Then, the upper layer solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared FeCl₃ solution (0.5 mL, 0.1 %). After dark incubation at room temperature (23°C), the absorbance was recorded at 700 nm. The antioxidant activity of sample extract was expressed as IC₅₀ value (mg/mL), the extract dose required to cause a 50 % decrease of the absorbance.

DPPH radical scavenging activity test

The antioxidant activity of the extract was determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) test, as described earlier with little modifications²¹. The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/mL. In Brief, 100 µL of plant extract at different concentrations (0, 0.1, 0.8, 0.6, 0.4, 0.2 and 1 mg/mL) were mixed with 1 mL DPPH methanol solution and left in darkness at room temperature (25 °C). After 30 minutes, the absorbance of the mixture was then recorded at 517 nm.

The same procedure was repeated for the ascorbic acid and the calibration line was construed. All determinations were carried out in triplicate. The DPPH scavenging activity test of the plant extract was calculated and IC₅₀ (Concentration of sample required to scavenge 50 % of DPPH radicals) value was determined from this formula: % Inhibition = $100 \times [(A_0 - A_1) / A_0]$

Where: A₀ was the absorbance of the control (just DPPH solution) and A₁ was the absorbance for the tested sample.

Statistical Analysis

All results were carried out in triplicate, and presented as mean ± standard deviation. The amount of total flavonoid and phenolic contents and IC₅₀ values were obtained from linear regression analysis in the Microsoft Excel program (2007).

RESULTS

Qualitative Phytochemical Screening

In this study, the results of the phytochemical screening of the aqueous leaf extract of *S.*

pachycarpa reveals the presence of various bioactive compounds such as phenolic compounds and alkaloid. However, tannins, glycoside, triterpenes/steroids and saponins were found absent (Table 1).

Table 1: Phytochemical screening of aqueous extract of *Sesbania pachycarpa* leaves

Phytochemical	Results
Alkaloids	+
Phenolic compounds	+
Flavonoid	+
Tannins	-
Saponins	-
Triterpenes /steroids	-
Glycoside	-

+ = Present, - = Absent

Determination of total flavonoid content

Table 2 presents the total flavonoid content expressed in milligrams of rutin equivalent per 100 g of extract (mg ER 100 g⁻¹). According to table 1, it is noted that at the concentration of 0.01 mg / mL, there is an absorbance of the order of 0.01 ± 0.01 nm and at 0.1 mg /mL, the absorbance reaches 0.41 ± 0.26 nm. The rutin equivalent was recorded: 67.03 ± 2.78 mg per 100 grams of plant material with the aqueous extract of the leaves of *S. pachycarpa*.

Determination of total phenolic content

Table 3 presents the content of phenolic compounds expressed in milligrams of gallic acid equivalent per 100 g of extract (mg GaE 100 g⁻¹). According to the table below, it is noted that at the concentration of 0.02 mg / mL, we have an absorbance of the order of 0.09 ± 0.05 nm and at 0.1 mg /mL, the absorbance reaches 0.38 ± 0.01 nm. In gallic acid equivalent, 141.56 mg per 100 grams of plant material was recorded with the aqueous extract of the leaves of *S. pachycarpa*.

Table 2: Total flavonoid contents of aqueous extract of *Sesbania pachycarpa* leaves

	Rutin							SPLE	TFC
Conc.	0.1	0.08	0.06	0.04	0.02	0.01	0	1	67.03±2.78
Abs.	0.41	0.32	0.24	0.14	0.04	0.01	0.00	0.27	/
	±0.26	±0.02	±0.01	±0.02	±0.01	±0.01			

Values are expressed as mean of triplicate determinations ± standard deviation, TPC=Total Flavonoid content (mg rutin acid equivalent/100g plant extract), Abs. = absorbance at 510 nm, Conc. = concentration (mg/mL), SPLE= aqueous extract of *Sesbania pachycarpa* leaves

Table 3: Total phenolic contents of aqueous extract of *Sesbania pachycarpa* leaves

Gallic Acid							SPLE	TPC
Conc.	0.1	0.08	0.06	0.04	0.02	0.01	1	141.56±5.23
Abs.	0.38	0.28	0.23	0.15	0.09	0.05	0.52	/
	±0.01	±0.01	±0.01	±0.03	±0.05	±0.01	±0.05	

Values are expressed as mean of triplicate determinations ± standard deviation, TPC=Total phenolic content (mg gallic acid equivalent/100g plant extract), Abs. = absorbance at 765 nm, Conc. = concentration (mg/mL), SPLE= aqueous extract of *Sesbania pachycarpa* leaves

Ferric-reducing antioxidant power (FRAP) assay

Table 4 presents the Ferric-reducing antioxidant power (FRAP) activity test. From the results obtained (Table 4), we note that at the concentration of 0.1 mg /mL, there is a percentage inhibition of the order of $32.15 \pm 0.01\%$ and at 1 mg /mL, the percentage reaches $45.85 \pm 0.01\%$. The IC_{50} value for aqueous extract and ascorbic acid are 0.173 and 0.33 mg /mL respectively.

Radical scavenging activity by DPPH**Table 4: Ferric reducing power activity (%inhibition)**

Test samples	Concentrations (mg/mL)						
	0.1	0.8	0.6	0.4	0.2	1	IC_{50}
Aqueous extract (%)	32.15	35.26	38.45	40.17	43.16	45.85	0.17
	± 0.01	± 0.04	± 0.02	± 0.01	± 0.02	± 0.01	± 0.01
Ascorbic acid (%)	38.64	40.51	45.21	48.22	51.24	55.45	0.33
	± 0.04	± 0.03	± 0.01	± 0.03	± 0.02	± 0.01	± 0.04

IC_{50} (mg/mL), aqueous extract = aqueous extract of *Sesbania pachycarpa* leaves

Table 5: DPPH scavenging activity (%inhibition)

Test samples	Concentrations (mg/mL)						
	0.1	0.2	0.4	0.6	0.8	1	IC_{50}
Aqueous extract (%)	49.23	52.24	57.02	66.3	66.35	47.54	0.921
	± 0.01	± 0.02	± 0.01	± 0.042	± 0.03	± 0.04	± 0.02
Ascorbic acid (%)	55.87	62.55	69.92	80.07	90.14	94.23	1.099
	± 0.03	± 0.06	± 0.02	± 0.03	± 0.01	± 0.04	± 0.01

IC_{50} (mg/mL), aqueous extract = aqueous extract of *Sesbania pachycarpa* leaves

DISCUSSION

From the results obtained in table 1, the phytochemical screening of *S. pachycarpa* leaves revealed the presence of as phenolic compounds and alkaloid. However, tannins, glycoside, triterpenes/steroids and saponins were found absent. In literature, the phytochemical screening of extract ethanol leaf of *S. pachycarpa* leaves revealed the presence of coumarins, flavonoids,

The results of the Radical scavenging activity by DPPH activity test are shown in table 5. It appears from this table that for concentrations of 0.1 and 0.2 mg /ml, the extract revealed DPPH inhibition percentages of $49.23 \pm 0.01\%$ and $52.24 \pm 0.01\%$ values respectively lower than the standard, ascorbic acid which has percentages of the order of $55.87 \pm 0.03\%$ and $62.55 \pm 0.06\%$ at the same concentrations. The IC_{50} value for aqueous extract and ascorbic acid are 0.921 and 1.099 mg /ml respectively with strong antioxidant activity.

tannins, phenolic compounds, alkaloids, saponosides, and triterpens and/orsteroids¹³.

The method is based on the ability of flavonoid to chelate aluminum chloride and form a yellowish complex. They contain free hydroxyl groups (OH), in positions 3 and 5, which are capable of forming complexes with the group CO, in the presence of aluminum chloride. The intensity of the coloring is proportional to the amount of flavonoid in the extract²².

The Folin-Ciocalteu reagent, a mixture of phosphotungstic ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) and phosphomolybdic acid ($\text{H}_3\text{PMO}_{12}\text{O}_{40}$), is reduced in the presence of phenolic to a blue mixture of tungsten oxide (W_8O_{23}) and molybdenum (Mo_8O_{23}). The blue coloration produced is proportional to the levels of phenolic compounds present in plant extracts²³. Table 2 shows the variation of mean absorbance with concentration of Gallic acid. Phenolic compounds are promising candidates as potential protectors against oxidative stress by different mechanisms of action, such as free radical scavenging²⁴, transition metal chelation²⁵. This potency measures the ability of an antioxidant to reduce ferric iron Fe^{3+} (FeCl_3) to ferrous iron Fe^{2+} (FeCl_2) in the presence of a chromogenic agent potassium ferricyanide $\text{K}_3[\text{Fe}(\text{CN})_6]$. This results in the change from yellow color of potassium ferricyanide to a blue green color whose intensity depends on the reducing power of the antioxidant²⁶.

Therefore, Fe^{2+} can be assessed by measuring and monitoring the increase in density of the blue color in the reaction medium at 700 nm. It is noted that even at low concentrations, the extract shows a high percentage of inhibition, which allows us to deduce that the phenolic compounds contained in the aqueous extract of the leaves of *S. pachycarpa* are very effective as antioxidants. The antiradical activities of herbal extract were assessed using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity test. DPPH is a free, stable radical, purple in color as a reagent, the intensity of which is measured at 517 nm²⁷. This results showed that with the increasing of the concentration, the DPPH free radical scavenging potential observably increased. Roughly 0.8mL/mL of the extract could scavenge about $66.35 \pm 0.03\%$ of the free radicals. The IC_{50} of DPPH scavenging activity was recorded as 0.921 mg /mL. The antioxidant works by transferring an electron or hydrogen atom to a hydrogen acceptor (DPPH). This is accompanied by the reduction of 1, 1'-diphenyl-1-picrylhydrazyl to 1, 1'-diphenyl-1-picrylhydrazine and a change in color to yellow²⁸. The high phenol and flavonoids contents of leaves extract may cause high antioxidant activity of this plant. According to these results, the activity of *S. pachycarpa* is not only attributed to their richness in phenolic and flavonoid but also to the chemical structure of phenolic compounds and the availability of hydroxyl groups (OH) which attributes their capacity transfer electrons or donate hydrogens²⁹.

CONCLUSION

S. pachycarpa is a reservoir of secondary metabolites that can be considered as alternative sources of powerful natural antioxidants used in pharmacology. Indeed, the leaf extract showed a content of interesting phenolic compounds correlated with good antioxidant activity with $\text{IC}_{50} = 0.921$ (DPPH) and 0.173 (FRAP) mg / mL which are slightly lower than that of the standard (ascorbic acid), with an IC_{50} value of 1.099 and (0.33 mg /mL) respectively. Phytochemistry test has revealed phenolic compounds, flavonoids and alkaloids in this extract explaining the strong antioxidant activity observed. Additional work is required to isolate these bimolecular responsible for the pharmacological effect and to purify them in order to understand the molecular mechanism.

AUTHORS' CONTRIBUTIONS

JV designed hypothesis, conceived, wrote the paper, and critically reviewed the manuscript and SDS was responsible for statistical work, calculations to manuscript proofing and carried out discussion of the present study.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest in the subject matter or materials discussed in this study.

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