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Plant biology and pathology/Biologie et pathologie végétales

Assessment of polyphenolic compounds, in vitro antioxidant and anti-inflammation properties of *Securidaca longepedunculata* root barksFrançois Nsemi Muanda^{a,*}, Amadou Dicko^a, Rachid Soulimani^b^a Chemistry Laboratory and methodology for Environment, Paul-Verlaine University, 1, boulevard Arago, 57078 Metz, France^b Neurotoxicology Alimentary and Bioactivity Laboratory, University Paul-Verlaine, BP 4102, 57040 Metz, France

ARTICLE INFO

Article history:

Received 26 April 2010

Accepted after revision 6 July 2010

Keywords:

Polyphenolic compounds

Flavonoids

Free radicals

RP-HPLC

Medicinal plants

Antioxidant activity

ABSTRACT

The present study was carried out to evaluate the antioxidant activities of the root bark extract of *Securidaca longepedunculata*. This plant material is commonly used in folk medicine in several parts in the world. The bark extracts of *S. longepedunculata* were evaluated for their total phenols, flavonoids, anthocyanins, tannins content and total antioxidant capacity. The compounds were identified and quantified both by RP-HPLC and UV spectrophotometer; the antioxidant capacity was assessed by ABTS and DPPH tests and expressed as IC₅₀. The total phenolic compounds determinate was 9.86 mg gallic acid equivalents/g dw, the total flavonoid contents was 5.85 mg catechin equivalents/g dw, the total anthocyanin contents was 0.032 mg cyanidin-3-glycosyl equivalents/g dw and the condensed tannins content were 1.03 mg catechin equivalents/g dw. The major compound identified using RP-HPLC was quercetin (0.98 mg/ml). The IC₅₀ value reached 5.5 μg/ml, revealing that the root barks of *S. longepedunculata* have a very high antioxidant and anti-inflammation properties.

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1. Introduction

Around the world, people having difficulties accessing modern medicine use traditional medicine as an alternative to conventional treatments. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Numerous investigations prove that among the secondary metabolites of medicinal plants, polyphenolic compounds exhibiting pharmacological properties take more and more importance. Several classes of phenolic compounds exist, including phenols, phenolic acids, phenyl propanols, flavonoids, minor flavonoids, tannins and quinines [1]. Recent studies reveal that they act as anti-allergenic, antimicrobial, anti-inflammatory, vasoprotector and anti-tumour agents. Some of the potential health benefits of

these polyphenolic substances have been attributed to their antioxidant capacity due to their free radical scavenger properties [2,3].

Much research takes a great interest in medical plants for their phenolic compounds concentrations and related total antioxidant potential [4–9]. The rich identifying of phytochemicals with the capacity to interfere carcinogenesis has received considerable attention. Oxidation and inflammation are well recognized to be closely linked. Therefore, the determination of antioxidation functions has been proposed as a good indicator for screening or evaluating plants for medical properties [10].

In this spirit, the present study was carried out to characterize the root barks of *Securidaca longepedunculata* with regard to their phenolic contents, anti-inflammation and antioxidant properties. *S. longepedunculata* is widely used for several diseases, in America (Venezuela) traditional medicine use *S. longepedunculata* dried barks for the treatment of epilepsy [11]. In Africa (Ghana, Nigeria) the plant decoction is prescribed by Ghanaian healers to treat

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asthma and other diseases associated with smooth muscle contraction [12] and as antinociceptive and antidepressant [13].

Moreover, the traditional use of *S. longepedunculata* root bark against the hepatic infection has been reported and their anti-allergenic effects were verified in vivo [14,15].

To our knowledge, few investigations have been made about the antioxidant properties of *S. longepedunculata* root bark, although that this medicinal plant is widely used by African traditional healers. So, the aims of this study were: (a) to determine the total phenolic, the total flavonoid, the total anthocyanin, and the condensed tannin contents, (b) to identify and quantify some of them by RP-HPLC methods; and (c) to evaluate the antioxidant, anti-inflammation properties of the extracts of the *S. longepedunculata* root bark using an UV-spectrophotometer. It was hoped to confirm and justify the use of this plant material in folk medicine.

2. Materials and methods

2.1. Chemicals

All the chemicals used were analytical grade. 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) (ABTS), 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH), gallic acid, folin-Ciocalteu's phenol reagent, aluminium chloride, catechin, *p*-coumaric acid, rutin, procatechiuc acid, vitamin C, caffeic acid, isovitexin, vitexin, chlorogenic acid, catechin, quercetin, quercetin dihydrat, quercetin-3- β -D-glucosyl, epicatechin, Kuromanin chloride, cyaniding chloride were purchased from Across organics (Geel, Belgium). Sodium carbonate, sodium nitrite, chlorhydric acid, ethyl acetate sodium, sodium sulphate anhydrous, ammonium phosphate, acetonitrile, methanol, interferon-gamma (IFN- γ), polysaccharide of salmonella typhimurium (LPS), sulfanilamide, NEDA (N-(1-naphtyl) ethylenediamine dihydroxy chloride), vanillin reagent, n-hexane were obtained from Sigma and Roth (Strasbourg, France).

2.2. Procurement and preparation of plant materials

Dried *S. longepedunculata* root bark (Polygalaceae) was obtained from Nigeria, and the botanical identification was carried out by Professor Max Henry, from the Botanic and Mycology Laboratory, Nancy University, France. A Voucher specimen has been kept in our Laboratory for future reference. All plant materials were dried at room temperature and powdered and sifted in a sieve (0.750 μ m).

2.3. Sample extractions

2.3.1. Total phenolic compounds (TPC), total flavonoid compounds (TFC), and total anthocyanin compounds (TAC)

Total phenolic compounds (TPC), total flavonoid compounds (TFC), and total anthocyanin compounds (TAC) were extracted from the powders as described by a previously reported method [6]. Two grams of powdered sample were extracted twice with 10 ml of cold aqueous methanol solution (50%). The two volumes were combined,

made up to 40 ml, centrifuged at 1532 \times g for 20 minutes and transferred in small sample bottles and stored at +4 $^{\circ}$ C in the dark until analysis. The analysis was carried out with a UV-Vis spectrophotometer (Varian Cary 50 scan).

2.3.2. Preparation of condensed tannins extracts (CT)

Samples for the CT were extracted from the powders as described by a previously reported method slightly modified [16]. One gram of powder was extracted twice with 20 ml of n-hexane during 20 minutes, filtered and the remaining powder was dried at 35 $^{\circ}$ C under vacuum for 2 h. The powder was stored at +4 $^{\circ}$ C until analyzes. A UV-vis spectrophotometer Varian Cary 50 Scan was used for the analyzes.

2.3.3. Extraction of polyphenol compounds for RP-HPLC analysis

Polyphenols were extracted according to the method described by a previously reported method slightly modified [17]. A fresh sample (0.4 g) was extracted with 2 \times 5 ml of aqueous methanol (20/80) with intermittent shaking (2 minutes) on vortex mixer for 30 minutes. The sample was centrifuged at 1532 \times g for 20 minutes at 20 $^{\circ}$ C. The supernatant was taken into a 10 ml volumetric flask. The extract is stable for 24 h if stored at 4 $^{\circ}$ C. The extracts were filtered through a 0.45 μ m (PTFE) filter before the injection was made. The analyzes were carried out on a RP-HPLC with a Waters 600E pump coupled to a Waters 486 UV visible tunable detector and equipped with a 20 μ l injection loop and an Alltech Intertsil ODS column (RP C18 column size 4.6 mm \times 150 mm; particle size, 5 μ m).

2.4. Determination of total phenolic compounds (TPC)

The Folin-Ciocalteu method [4] was used to measure the total phenolic compounds. To a sample (100 μ l), distilled water was added to make 2 ml (Eppendorff tube), followed by 1 ml of Folin Ciocalteu reagent 1N and sodium carbonate (20%). After 40 minutes at room temperature, absorbance at 725 nm was read on a spectrophotometer against a blank that contained methanol instead to sample. The results were compared to a gallic acid calibration curve, and the total phenolic compounds were determined as mg gallic acid equivalents (GAE mg/g dry weight (dw)). Determination of each sample was performed in triplicate.

2.5. Determination of total flavonoid compounds (TFC)

The flavonoid contents were measured according to a colorimetric assay [18]. A 250 μ L of standard solution of catechin at different concentrations or appropriately diluted samples was added to 10 ml volumetric flask containing 1 ml of distillate water. At zero time, 75 μ l of NaNO₂ (5%) was added to the flask. After 5 minutes, 75 μ l of AlCl₃ (10%) was added. At 6 minutes, 500 μ l of NaOH (1N) was added to mixture. Immediately, the solution was diluted by adding 2.5 ml of distillate water and mixed thoroughly.

Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. Total flavonoid compounds in medicinal plant were expressed as

mg catechin equivalents (CE mg/g dw). Samples were analyzed in three replications.

2.6. Evaluation of total anthocyanin compounds (TAC)

The total anthocyanin compound of the samples was estimated using a UV-spectrophotometer by the pH-differential method [6,19]. Two buffer systems, potassium chloride buffer, pH 1.0 (0.0025 M) and sodium acetate buffer, pH 4.5 (0.4 M) were used. Briefly, 400 μ l of extract was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm. Absorbance (ΔA) was calculated as: $\Delta A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.0}}$ monomeric anthocyanin pigment concentration in the extract was calculated and expressed as equivalent cyaniding-3-glucoside (mg/l): $\Delta A \times MW \times Df \times 1000 / (Ma)$ with ΔA : difference of absorbance, Mw: molecular weight for cyaniding-3-glucoside (449.2); Df: the dilution factor of the samples, and Ma: molar absorptivity of cyaniding-3-glucoside (26,900). Results were expressed as mg of cyaniding-3-glucoside equivalents in 100 g of dried sample.

2.7. Determination of condensed tannins (CT)

The condensed tannins content was estimate using a previously reported method [16] with some modifications. Briefly, an aliquot of 0.5 g of powder obtained after lixiviation (n-hexane) was placed in a centrifuge tube and 15 ml of 1% HCl in methanol was added to each sample. Each tube was vortexed and placed in a water bath at 35 °C with constant shaking for 20 minutes and vortexing every 5 minutes.

After incubation, the tubes were centrifuged (1532 \times g) and the supernatants were extracted. Aliquots of the supernatants (100 μ l) were placed in two separate assay tubes, one for the sample determination and the other for blank determination.

Samples and blanks were incubated for exactly 20 minutes after adding 5 ml of vanillin reagent (0.5 g of reagent and 200 ml of 4% HCl methanol) to samples and 4% HCl in methanol to the blanks. After 20 minutes, absorbance was read at 500 nm from each sample and blank using UV-spectrophotometer Varian Cary 50.

Samples absorbance was rectified with the blank standard and compared against a standard curve made with catechin. Results were expressed as mg catechin equivalent/g (mg CE/g) of lixiviating sample. The samples were analyzed at least for three replications.

2.8. RP-HPLC analysis

The RP-HPLC analyzes were performed according to the modified method described by a previously reported method [20], using an elution gradient of three mobile phases; solvent A: 50 mM ammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) pH 2.6 (adjusted with phosphoric acid), solvent B: (80:20 (v/v)) acetonitrile/solvent A, and solvent C: 200 mM of phosphoric acid pH 1.5 (pH adjusted with ammonium hydroxide). The gradient profile was linearly changed as follows (total 60 minutes): 100% solvent A at

zero minutes, 92% A/ 8% B at 4 minutes, 14% B/ 86% C at 10 minutes, 16% B/ 84% C at 22.5 minutes, 25% B/ 75% C at 27.5 minutes, 80% B/ 20% C at 50 minutes, 100% solvent A at 55 minutes, 100% A at 60 minutes. The flow rate was set at 1 ml/minute at room temperature. Before the injection, the samples were filtered through a 0.45 μ m PTFE syringe tip filter. Detection was done at 280 and 320 nm. Polyphenolic external standards were prepared by dissolving 2 mg/ml.

In each sample, a polyphenolic compound was identified by matching its retention time to the corresponding external standard and the concentration of the polyphenolic compound was calculated by comparing their peak areas. The samples were analyzed at least three replications.

2.9. Antioxidant activities

Two tests have been used to determine the total antioxidant capacity, the DPPH and the ABTS tests.

2.9.1. DPPH radical scavenging test

The DPPH radical scavenging activity was evaluated according to the method slightly modified described by others [21,22]. 1 ml of 100 μ M DPPH solution in methanol was mixed with 1 ml of plant extract. The reaction mixture was incubated in the dark for 20 minutes, and the optical density was recorded at 517 nm against the blank. For the control, 1 ml of DPPH solution in methanol was mixed with 1 ml of methanol and optical density of solution was recorded after 20 minutes.

The decrease in optical density of DPPH in samples with regard to control system was used to calculate the antioxidant activity as a percentage inhibition (%PI) of DPPH radical, $\%IP = [(At_0 - At_{20}) / (At_0)] \times 100$ where At_0 : absorbance of the test sample after zero minutes and At_{20} : absorbance of control after 20 minutes. Each assay was carried out in triplicate.

From a plot of concentration against %IP, a linear regression analysis was performed to determine the IC_{50} value for each extract. The DPPH radical scavenging activity of phenolic compounds was expressed as IC_{50} value in μ g/ml of dry weight. A low IC_{50} value represents a high antioxidant activity.

To 2.90 ml of an aqueous methanol solution (50%) of 100 μ M of DPPH, 100 μ l of the plant extract solution was added. The mixture was incubated in a 20 °C water bath under restricted light for 40 min. A control (100 μ l of 50% methanol and 2.90 ml of DPPH solution) was run with each series of samples. The reduction of absorbance at 517 nm was measured 40 min later. The radical solution was prepared daily. The DPPH radical scavenging activity of phenolic compounds was expressed as mg/g dw of Vitamin C Equivalents (VCE) in 40 minutes.

2.9.2. ABTS radical scavenging test

The method developed by a previous one [23] (slightly modified) had been used in this experiment. 1.0 mM of AAPH solution was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution 100 mM potassium phosphate buffered (pH 7.4) containing 150 mM NaCl. The mixture was heated in a water bath at 68 °C for 20 minutes. The concentration of the resulting

blue-green ABTS^{•+} (radical cation solution) was adjusted to an absorbance of 0.65 ± 0.02 at 734 nm. The sample solution of 60 μ l was added to 2.94 ml of the resulting blue-green ABTS radical solution.

The mixture, protected from light, was incubated in a water bath at 37 °C for 20 minutes. A control solution (60 μ l of methanol and 2.94 ml of ABTS^{•+}) was monitored with each series of samples. Then the decrease of absorbance at 734 nm was measured. The stable ABTS radical scavenging activity of the phenolic compounds in the extract was expressed as mg/g dry plants part powders of Vitamin C Equivalents (VCE) in 20 minutes. All radical stock solutions were prepared fresh daily.

2.10. Anti-inflammatory activity by nitrite assay

The test used to assess the potential anti-inflammatory activity of molecules consisted of evaluating their capacity to inhibit NO production in activated macrophages [24]. Released nitrite (NO_2^-) in the culture medium was measured as an indicator of NO production according to the colorimetric test based on the Griess reaction. Briefly, 1 mL of plant extract was mixed with 1 mL of Griess reagent at room temperature for 30 minutes. The nitrite concentration was determined by measuring the absorbance at 548 nm using a standard curve of NaNO_2 .

The results were expressed as percentage of NO production compared to the control as follows:

$$\% \text{ Inhibition} = 100 \times \frac{[\text{NO}_2^-]_{\text{control}} - [\text{NO}_2^-]_{\text{Ex.}}}{[\text{NO}_2^-]_{\text{control}}}$$

where $[\text{NO}_2^-]_{\text{control}}$ is the concentration of nitrite released without addition of the extract, and $[\text{NO}_2^-]_{\text{Ex}}$ the concentration of nitrite released by the cells in presence of the plant extract.

2.11. Statistical analysis

Results are presented as mean \pm standard Error; statistical analysis of experimental result was based on analysis of variance. Correlation analyzes of antioxidant activity versus the total polyphenolic contents were carried out using the Statview[®] 4.5 statistical package (Abacus Concepts, Inc). Significant difference was statistically considered at the level of $P < 0.001$.

3. Results and discussion

3.1. Total phenolic contents

The root of *S. longependiculata* is mainly composed of polyphenolic (9.86 mg GAE/g dw) and flavonoid compounds (5.85 mg CE/g dw). The amount of total tannin and total anthocyanin compounds are weak 1.03 mg CE/g dw and 0.032 mg CgE/g dw, respectively (Fig. 1).

3.2. HPLC analysis

The RP-HPLC analysis of the extracts were conducted in two solvents: water (WE: water extract) and methanol-

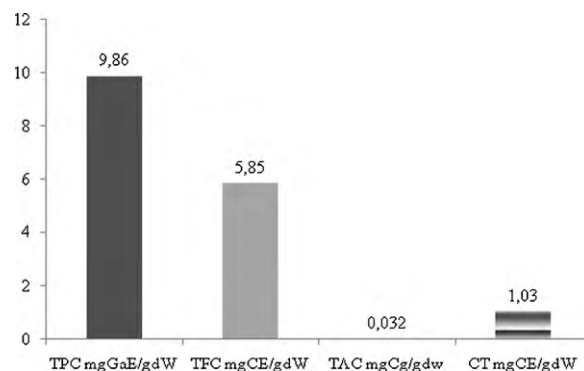


Fig. 1. Phenolic compounds of the root barks of *S. longependiculata*. Gallic acid equivalent (GAE); Catechin equivalent (CE); Total phenolic compounds (TPC); Total flavanoid compounds (TFC); Total anthocyanin compounds (TAC); Condensed tannins (CT).

water (MWE: methanol-water extract). Figs. 2 and 3 show the profiles of the injection of the extracts detected at 280 and 320 nm. In Table 1, the retention time of external standards were reported and their corresponding concentration in the extracts were expressed in mg/ml.

Quantitative and qualitative analysis show that water is not the best extract solvent for phenolic compounds, that is in concordance with previously studies, which report that an aqueous alcohol solvent is the best one to extract phenolic compounds from plant materials [20,22]. The most important compound identified in WE was quercetin (0.61 mg/ml) and the less one was gallic acid (0.03 mg/ml). In the MWE, quercetin (0.98 mg/ml) and apigenin (0.68 mg/ml) were the major compounds identified. Gallic acid (0.02 mg/ml) followed by acid p-coumaric acid (0.05 mg/ml) and quercetin glucosyl (0.05 mg/ml) were minor. The samples were analyzed at least four replications.

3.3. Antioxidants activities

ABTS and DPPH tests revealed that *S. longependiculata* root barks had good scavenging antioxidant activities. Little variation had been observed ABTS test value express as mg VCE/ g dw is 1.351 and DPPH value is 9.48 mg VCE/g dw. To compare the antioxidant capacity of *S. longependiculata* root barks extract with others plants previously described in the literature, this value was reported as IC_{50} (μ g/ml) (Table 2).

These results show that the *S. longependiculata* root barks extract present a good antioxidant capacity.

Because its IC_{50} value ($\text{IC}_{50} = 5.5 \mu\text{g/ml}$) is greater than those of some others plant extracts, well known for their antioxidant properties such as *Litchi* seeds ($\text{IC}_{50} = 4.8 \mu\text{g/ml}$), *Anogeissus dhofarica* A.J. Scott ($\text{IC}_{50} = 4.5 \mu\text{g/ml}$), *Allophylus rubifolius* Hoschst ex A. Rich Engl. ($\text{IC}_{50} = 7.1 \mu\text{g/ml}$), orange juice and orange wine ($\text{IC}_{50} = 0.31$ & 0.46 mg/ml) [22,25,26].

3.4. Relation between phenolic compounds and antioxidant capacity

In Fig. 4, we found that phenolic compounds were major contributors to antioxidant activity, because

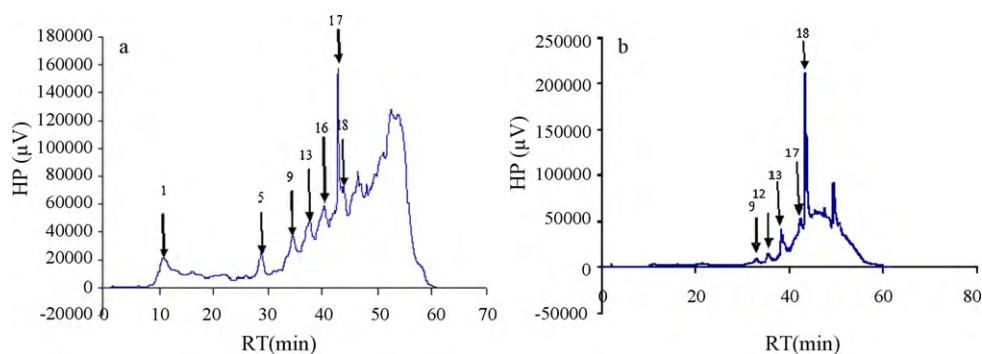


Fig. 2. a: Chromatogram of the *S. longependunculata* root bark extract (WE) (280 nm). 1: gallic acid; 5: caffeic acid; 9: *p*-coumaric acid; 13: quercetin glucosyl; 16: cinnamic acid; 17: quercetin; 18: apigenin. b: Chromatogram of the *S. longependunculata* root bark extract (WE) (320 nm). 9: *p*-coumaric acid; 12: rutin; 13: quercetin glucosyl; 16: cinnamic acid; 18: apigenin.

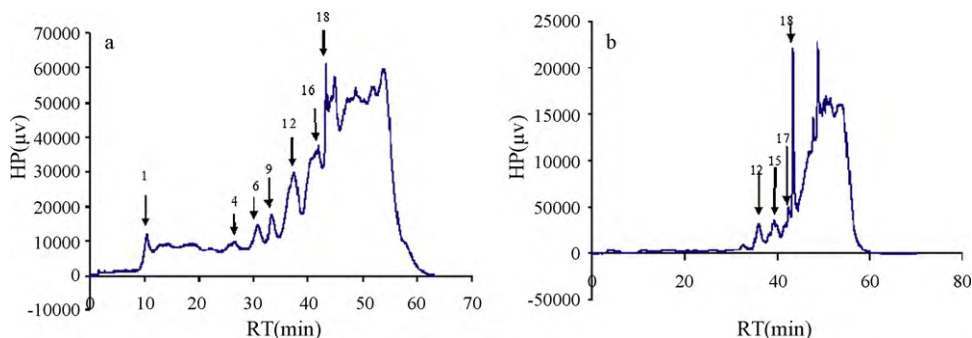


Fig. 3. (a) Chromatogram of the *S. longependunculata* root bark extract (MWE) (280 nm). 1: gallic acid; 4: chlorogenic acid; 6: epicatechin; 9: *p*-coumaric acid; 12: rutin; 16: cinnamic acid; 18: apigenin. (b) Chromatogram of the *S. longependunculata* root bark extract (MWE) (280 nm). 12: rutin; 15: quercetin dihydrat; 17: quercetin; 18: apigenin.

Table 1

Compounds of *S. longependunculata* root bark and their concentrations in WE and MWE (280 & 320 nm).

Compound numbers	Name of compounds	Rt (min)	WE (mg/ml)	MWE (mg/ml)
1	Gallic acid	11.98 ± 0.22	0.03 ± 0.01	0.02 ± 0.01
3	Chlorogenic acid	26.51 ± 0.48	nd	0.04 ± 0.01
5	Caffeic acid	28.92 ± 0.29	0.05 ± 0.02	nd
6	Epicatechin	31.45 ± 0.55	nd	0.06 ± 0.01
9	<i>p</i> -coumaric acid	33.25 ± 0.36	0.15 ± 0.03	0.05 ± 0.02
12	Rutin	37.02 ± 0.36	0.08 ± 0.01	0.12 ± 0.03
13	Quercetin glucosyl	37.71 ± 0.18	0.17 ± 0.05	nd
15	Quercetin dihydrat	39.35 ± 0.58	nd	0.05 ± 0.01
16	Cinnamic acid	41.68 ± 0.65	0.06 ± 0.01	0.21 ± 0.07
17	Quercetin	42.43 ± 0.30	0.61 ± 0.05	0.68 ± 0.01
18	Apigenin	43.80 ± 0.29	0.23 ± 0.02	0.98 ± 0.06

MWE: methanol water extract; WE: water extract; nd: not determinate; Rt: retention time.

antioxidant activity and phenolics TPC, TFC and TAC showed a good correlation with coefficient correlations of $R^2 = 0.92$, $R^2 = 0.94$ and $R^2 = 0.87$, respectively. However, antioxidant capacity and condensed tannin showed a very weak relationship with a correlation coefficient of $R^2 = 0.04$. Although the majority antioxidant capacity of plants is not only represented by phenolics, flavonoids, anthocyanins or tannins which have a strong antioxidant potential, but is also due to other compounds such as vitamin [22].

3.5. Anti-inflammatory properties of phenolic compounds

The anti-inflammatory activity of MWE was studied *in vitro* for their inhibitory effects on chemical mediators release (LPS/ IFN γ -induced NO production) from macrophages. Activated macrophages produce large amounts of chemical mediators that indicate inflammation.

NO, a bioactive free radical, is one of these critical mediators which is produced by inducible NO synthase (iNOS) in inflammatory macrophages when stimulated

Table 2
Antioxidant activity in vitro analysis.

Sample	ABTS test	DPPH test	
<i>S. longependunculata</i> root barks extract	VCE mg/ g dw 11.53 ± 0.22	mg VCE/g dw 7.47 ± 0.10	IC ₅₀ (μg /ml) 5.50 ± 0.25

IC₅₀: concentration of a polyphenolic in mg/ml required to quench the free radicals by 50%; dw: dry weight; Vitamin C equivalent: VCE.

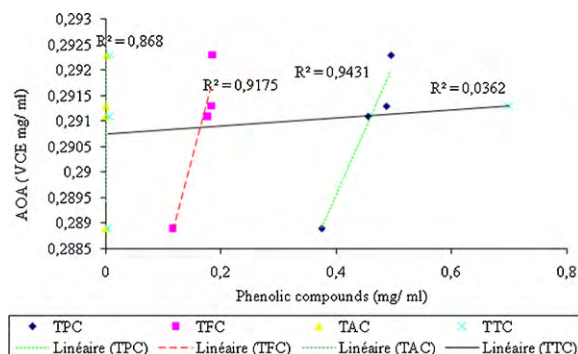


Fig. 4. Relationship between phenolic compounds and antioxidant activities. Antioxidant activities (AOA); Gallic acid equivalent (GAE); Catechin equivalent (CE); Total phenolic compounds (TPC); Total flavanoid compounds (TFC); Total anthocyanin compounds (TAC); Condensed tannins (CT).

with LPS/IFN γ mixture [24]. Excessive production of NO is indicated both in chronic and acute inflammation [27].

In fact, NO production induced by LPS/IFN γ through iNOS induction may reflect the degree of inflammation and may provide a measure for assessing the effect of the extract/fractions on the inflammatory process.

As shown in Fig. 5, the addition of MWE at different concentrations (20, 50, 100 and 150 μ l) significantly reduced NO production, resulting in 18.08, 25.65, 45.67 and 51.35% inhibition of NO production in macrophages stimulated with LPS/IFN γ , respectively. These results show a significantly reduced NO production in a dose-dependent manner. Similarly to our results, it has been demonstrated in several studies that extract rich in flavonoids and proanthocyanidins inhibited NO production in RAW 264.7 macrophages [28–30]. These results seem to indicate that phenolic compounds present in the extracts are responsible for the anti-inflammatory activity.

The anti-inflammatory activity correlates positively with the radical-scavenging activity and the highest

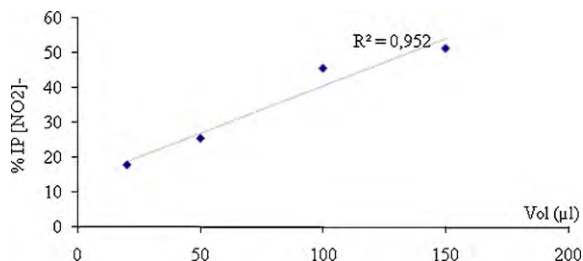


Fig. 5. Relationship between inhibition percentage of NO₂⁻ and the concentration (volume) of [NO₂]⁻.

antioxidant activity was correlated with the highest total phenol content and/or the highest anti-inflammatory activity of the tested extract/fraction. These results are consistent with those of Ojewole [15] and Akinmoladun et al. [31].

In this study, for the first time, phenolic contents and antioxidant activity of the root barks of *S. longependunculata* have been examined. The results indicated that from all phenolic compounds identified in the root barks extracts of *S. longependunculata*, quercetrin was the most abundant phenolic compound. The root barks extracts scavenge for the super oxide radicals and anti-inflammation properties in vitro.

Our results show an IP superior to 80%. In conclusion, we can say that our results further support the view that *S. longependunculata* root barks are promising sources of natural antioxidants and can be seen as potential sources of useful drugs.

Acknowledgments

This work was supported by a grant from the Ministry of Scientific Research of Republic Democratic of Congo (No. 132.49/060/KM/07). Mr. Frederic Desort (Neurotoxicology Alimentary and Bioactivity Laboratory). University P. Verlaine-Metz, BP 4102, 57040, Metz (France) is also acknowledged for technical assistance.

References

- [1] J.B. Harbone, *Phytochemical methods, a guide to modern techniques of plant analysis*, Third ed., Chapman and Hall, London, 1998, pp. 4–55, 150–160, 235–236.
- [2] S.C. Gordona, M.D. Sonja, M.C. Jasna, T.T. Vesna, Antioxidant proprieties of marigold extracts, *Food Res. Int.* 37 (2004) 643–650.
- [3] K. Chitindingu, A.R. Ndhkala, C. Chapano, M.A. Benhura, M. Muchuweti, Phenolic compound content, profiles and antioxidant activities of *Amaranthus hybridus* (Pigweed, Brachiaria Brizantha) (Upright Brachiaria) and *Panicum maximum* (Guinea grass), *J. Food Biochem.* 31 (2006) 206–216.
- [4] B. Dzingira, M. MuChuweti, T. Murenje, C. Chidewe, M.A.N. Benhura, L.S. Chagonda, Phenolic content and phospholipids peroxidation inhibition by methanolic extracts of two medicinal plants: *Elionurus muticus* and *Hypoxis hemerocallidea*, *Afr. J. Biochem. Res.* 1 (2007) 137–141.
- [5] C. Mariani, A. Bracca, S. Vitalini, N. De Tommasi, F. Visioli, G. Fico, Flavonoid characterization and in vitro antioxidant activity of *Aconitum anthoral* (Ranunculaceae), *Phytochemistry* 69 (2008) 1220–1226.
- [6] M.F. Abu Bakar, M. Mohamed, A. Rahmat, J. Fry, Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*), *Food Chem.* 113 (2009) 479–483.
- [7] P. Nuntanakorn, B. Jiang, H. Yang, M. Cervantes-Cervantes, F. Kronenberg, E.J. Kennelly, Analysis of polyphenolic compounds and radical scavenging activity of Four American Actaea species, *Phytochem. Anal.* 18 (2007) 219–228.
- [8] F. Shariffar, G. Dehghn-Nudeh, M. Mirtajaldini, Major flavonoids with antioxidant activity from *Teucrium polium* L., *Food Chem.* 112 (2009) 885–888.
- [9] E. Pastor-Cavada, R. Juan, J.E. Pastor, M. Alaiz, J. Vioque, Antioxidant activity of seed polyphenols in fifteen wild Lathyrus species from south Spain, *LWT Food Sci. Technol.* 42 (2009) 705–709.
- [10] T. Tzungu-Hsu, T. Po-Jan, H. Su-Chen, Antioxidant and anti-inflammatory activities of several commonly used spices, *J. Food Sci.* 70 (2005), C 93–C97.
- [11] O. Heard, Contribution of the study of *Desmodium adscendens*: chemistry & pharmacology [Dissertation]. Ph.D., thesis, University of Tours, France, 1994.
- [12] N.C. Rakuambo, J.J.M. Meyer, A. Hussein, C. Huyser, S.P. Mdlatlose, T.G. Raidani, In vitro effect of medicinal plants used to treat erectile

- dysfunction on smooth muscle relaxation and human sperm, *J. Ethnopharmacol.* 105 (2006) 84–88.
- [13] R.A. Adebisi, A.T. Elsa, B.M. Agaie, E.U. Etuk, Antinociceptive and antidepressant like effects of *Securidaca longepedunculata* root extract in mice, *J. Ethnopharmacol.* 107 (2006) 234–239.
- [14] L.P.M. Dapar, C.J. Aguiyi, N.N. Wannang, S.S. Gyang, M.N. Tanko, The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats, *Afr. J. Biotechnol.* 6 (2007) 591–595.
- [15] J.A. Ojewole, Analgesic and hypoglycaemic effects of *Securidaca longepedunculata* (Fresen) root-bark aqueous extract, *Inflammopharmacology* 16 (2008) 174–181.
- [16] J.E. Villareal-Lozoya, L. Lombardini, L. Cisneros-Zevallos, Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoensis* (Wangenh) K. Koch] cultivars, *Food Chem.* 102 (2007) 1241–1249.
- [17] V. Sharma, A. Gulati, S.D. Ravindranath, V. Kumar, A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC, *J. Food Comp. Anal.* 18 (2005) 583–594.
- [18] D.O. Kim, O.K. Chun, Y.J. Kim, H.Y. Moon, C.Y. Lee, Quantification of polyphenolics and their antioxidant capacity in fresh plums, *J. Agric. Food Chem.* 51 (2003) 6509–6515.
- [19] J. Lako, V.C. Trenerry, M. Wahlqvist, N. Wattanapenpaiboon, S. Sotheeswaran, R. Premier, Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods, *Food Chem.* 101 (2007) 1727–1741.
- [20] N. F. Muanda, D. Koné, A. Dicko, R. Soulimani, C. Younos: Phytochemical composition and antioxidant capacity of three Malian medicinal plant parts. *ECAM.* (2009) 1–8, doi: 10.1093/nep109.
- [21] D.O. Kim, S.W. Jeong, C.Y. Lee, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, *Food Chem.* 81 (2003) 321–326.
- [22] R.G. Marwah, M.O. Fatope, R.A. Mahrooqi, G.B. Varma, H.A. Abadi, S.K.S. Al-Burtamani, Antioxidant capacity of some edible and wound healing plants in Oman, *Food Chem.* 101 (2007) 465–470.
- [23] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, *Food Chem.* 97 (2006) 654–660.
- [24] M. Pacheco-Sanchez, Y. Boutin, P. Angers, A.R.J. Gosselin Tweddell, Inhibitory effect of CDP, a polysaccharide extracted from the mushroom *Collybia dryophila*, on nitric oxide synthase expression and nitric oxide production in macrophages, *Eur. J. Pharmacol.* 555 (2007) 61–66.
- [25] K. Nagendra Prasad, B. Yang, S. Yang, Y. Chen, M. Zhao, M. Ashraf, Y. Jiang, Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*litchi sinensis* Sonn) seeds, *Food Chem.* (116) (2009) 1–7.
- [26] H. Kelebek, S. Selli, A. Canbas, T. Cabaroglu, HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv, *Kozan, Microchem. J.* 91 (2009) 187–192.
- [27] X. Terra, et al., Grape-seed procyanidins act as anti-inflammatory agents in endotoxin-stimulated RAW 264.7 Macrophages by Inhibiting NFκB signaling pathway, *J. Agric. Food Chem.* 55 (2007) 4357–4365.
- [28] R.M. Clancy, B. Varneika, J. Rediske, D. Stoyanovsky, D. Wink, S.B. Abramson, Thenitric oxide (N.O) paradox resolved by Angeli's salt: tissue injury depends upon NO redox state, *Arthritis Rheum.* 8 (1998), 41, S 143.
- [29] S.C. Ho, L.S. Hwang, Y.J. Shen, C.C. Lin, Suppressive effect of a proanthocyanidin-rich extract from longan (*Dimocarpus longan* Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells, *J. Agric. Food Chem.* 55 (2007) 10664–10670.
- [30] P.N. Diouf, T. Stevanovic, A. Cloutier, Study on chemical composition, antioxidant and anti-inflammatory activities of hot water extract from *Picea mariana* bark and its proanthocyanidin-rich fractions, *Food Chem.* 113 (2009) 897–902.
- [31] A.C. Akinmoladun, E.M. Obuotor, E.O. Farombi, Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants, *J. Med. Food* 13 (2010) 444–451.