https://doi.org/10.31925/farmacia.2020.1.16 **ORIGINAL ARTICLE**

FREE RADICAL SCAVENGING ACTIVITY AND TOTAL POLYPHENOL CONTENT OF *SECURIDACA LONGIPEDUNCULATA* ROOTS AND LEAVES EXTRACTS

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Abstract

Securidaca longipedunculata Fresen. is a widely used medicinal plant in most of the African countries. This paper aims to compare the polyphenolic composition and antioxidant activity of some extracts obtained from *S. longipedunculata* roots and leaves in different solvents: ethanol (E), petroleum ether (PE), ethyl acetate (EtOAc), and water (W). Polyphenols were analysed by spectrophotometric and chromatographic methods. Evaluation of antioxidant activity was performed by several *in vitro* methods (DPPH bleaching, FRAP, nitrite-induced auto-oxidation of haemoglobin, **i**nhibition of lipid peroxidation catalysed by cytochrome C). Our results revealed that the leaves were abundant in phenolic principles than the roots and the EtOAc was the appropriate solvent for their extraction. The EtOAc extracts exhibited the best antioxidant potential. Several polyphenols were determined in the leaves extracts for the first time: hyperoside, isoquercitrin, rutin, *p*-coumaric acid.

Rezumat

Securidaca longipedunculata Fresen. este o plantă medicinală utilizată pe scară largă în majoritatea țărilor africane. Această lucrare își propune să compare compoziția polifenolică și activitatea antioxidantă a unor extracte obținute din rădăcini și frunze de *S. longipedunculata* în diferiți solvenți: etanol (E), eter de petrol (PE), acetat de etil (EtOAc) și apă (W). Polifenolii au fost analizați prin metode spectrofotometrice și cromatografice. Evaluarea activității antioxidante a fost efectuată prin mai multe metode *in vitro* (DPPH, FRAP, autooxidarea hemoglobinei indusă de nitriți, inhibarea peroxidării lipidice catalizată de citocromul *c*). Rezultatele noastre au arătat că frunzele au fost mai bogate în principii fenolice decât rădăcinile, iar acetatul de etil a fost cel mai bun solvent pentru extracție. Extractele în EtOAc au prezentat cel mai bun potențial antioxidant. Polifenolii de tip hiperozidă, izoquercitrină, rutozidă, acid *p*-cumaric au fost determinați pentru prima dată în extractele obținute din frunze.

Keywords: *Securidaca longipedunculata*; roots and leaves extracts

Introduction

Securidaca longipedunculata Fresen. (Violet tree) belongs to the *Polygalaceae* family, being widely used in traditional African medicine as a supply of local health care [13]. According to scientific reports, this species has relevant applications in ethno-medicine, with multiple indications for human diseases (asthma, hepatic infection, coughs, epilepsy, malaria, tuberculosis, constipation, headache, rheumatism, stomach ache) [5, 6, 13]. Numerous studies have reported that roots extracts have antimicrobial, antioxidant, antidiabetic, anticonvulsant, anti-inflammatory, antimalarial, insecticidal, pesticidal properties [12, 15, 16, 19]. As far as we know, few investigations have been reported on leaves extracts, although this medicinal plant is widely used [1, 9, 10]. So, the purpose of this study was to compare the chemical composition of different extracts obtained by leaves and roots of *S. longipedunculata* species, as well as to investigate their antioxidant potential, for better characterization and exploitation of these plant materials.

Materials and Methods

Plant material and extraction procedure. Freshly collected *S. longipedunculata* (S) leaves and roots were dissected into small sizes and dried at room temperature. The voucher specimen number according to the herbarium (Ethnobotany unit) of the Department

of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria is NIPRD/H/6576. The dried materials were powdered using electric blender (Bosch). The powdered plant materials: roots (*radix*, R, 30 g) and leaves (*folium*, F, 15 g) were extracted two times with 95% ethanol (150 mL x 2) under reflux for one hour each time, followed by filtration, combination and evaporation in vacuum to give the 95% ethanol dry extracts (SR1: 1.184 g and SF1: 4.216 g). The dried ethanolic extracts (0.824 g SR1 and 3.173 g SF1 dry extract) were suspended in water (100 mL) and transferred into the separatory funnel, then were sequentially extracted with petroleum ether (PE,100 mL x 3) and ethyl acetate (EtOAc, 400 mL x 3) to give petroleum ether extracts (SR2: 0.144 g and FS2: 0.216 g dry extract), ethyl acetate extracts (SR3: 0.264 g and SF3: 0.455 g dry extract), and water extracts (SR4: 0.413 g and SF4: 2.502 g dry extract) after removing the solvent *in vacuum* [11]. The solutions used for the analysis were obtained by dissolving the dried extracts in methanol.

Chemicals, quantitative analysis and HPLC chromatographic conditions. Chemicals were obtained from Merck, Alfa-Aesar and Roth (Germany).

Determination of total polyphenolic content (TPC). The TPC of the extracts was determined by slightly modified Folin-Ciocâlteu method [20]. Each extract of *S. longipedunculata* roots and leaves was mixed with Folin- Ciocâlteu reagent and sodium carbonate solution. Standard calibration curve was made with gallic acid plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL and prepared in methanol: water (50:50, v/v). TPC values were determined using the equation and slope generated from the calibration curve ($R^2 = 0.999$) and the results were expressed as mg of gallic acid equivalent (GAE)/g dry extract [2, 17, 20].

DPPH radical-scavenging activity. Free radical scavenging activity of *S. longipedunculata* extracts was measured by DPPH method. The DPPH solution (0.1 g/L) in methanol was prepared and 2.0 mL was added to 2.0 mL of extract (or standard) at different concentrations (SR1: 37.5 - 131.25 µg/mL; SR2: 28.5 - 228 µg/mL; SR3: 19.8 - 69.3 µg/mL; SR4: 206.5 - 619.5 µg/mL; SF1: 62.5 - 218.75 µg/mL; SF2: 108 - 432; SF3 µg/mL: 8.53 - 59.71 µg/mL; SF4: 62.55 - 218.92 µg/mL). The absorbance values were recorded at 517 nm. The antioxidant ability of natural compounds is based on quenching of stable coloured radical DPPH. The IC_{50} values for DPPH radical scavenging ability were represented by the concentration at which the samples exhibited 50% inhibition of the DPPH free radical. The % DPPH inhibition was plotted against Log dose (concentration) to obtain the curve and the IC_{50} [4, 7].

FRAP Assay. The antioxidant capacity of the extracts was estimated by the spectrophotometrically ferric reducing/antioxidant power (FRAP) assay following the procedure of Benzie and Strain [3]. The FRAP method relies on the colour changing of a complex with Fe^{+3} ion, the TPTZ (2,4,6 tripyridyl-S-triazine) radical (colourless complex), by the reduction of the Fe^{3+} form of iron to Fe^{+2}-tri-pyridyltriazine (blue coloured complex), which is due to the action of the electron donation from antioxidants. Trolox was used as a reference. The colour change was correlated with the antioxidant capacity by measuring the absorbance at 450 nm. Using a calibration curve ($R^2 = 0.992$), the results were converted to µM Trolox equivalents/g dry extract.

Nitrite-induced auto-oxidation of haemoglobin. The nitrite-induced auto-oxidation of haemoglobin (Hb) was studied at 580 nm, the wavelength where the transformation of haemoglobin from oxyhaemoglobin (oxy-Hb) to methaemoglobin (met-Hb) can be observed. Thus, 40 µM oxy-Hb were mixed with 166 µM nitrite in the presence of extracts. The obtained curve was fitted with a sigmoidal equation and the inflection time (t_i) was calculated using Origin 8. The results are expressed in mg CAE (chlorogenic acid)/g material plant, using a calibration curve with a R^2 of 0.99 [8].

Inhibition of lipid peroxidation catalysed by cytochrome C. Liposomes were obtained by sonication of 5 mg/mL soy lecithin, in phosphate 10 mM, pH 7. The reaction was catalysed by 2 µM cytochrome C in the presence of extracts (8.3 µg/mL) and was monitored in time at 235 nm [14].

HPLC-MS methods. The analysis of phenolic compounds previously described was conducted with an Agilent 1100 HPLC Series system equipped with a degasser and quaternary gradient pump, a model G1311A diode array detector system, a Zorbax SB-C18 reverse-phase analytical column [2, 4, 7]. All compounds were identified by comparison of retention times and the MS spectra with those of the standards, using the same parameters and chromatographic conditions. In order to identify polyphenols such as epicatechin, catechin, siringic, gallic, protocatechuic and vanillic acids, another HPLC-MS method previously described was used [4, 7]. The polyphenol compounds were determined on their peak areas and compared to a calibration curve of their six corresponding standards [4, 7]. The results were expressed as mg/g dry extract.

Results and Discussion

The polyphenolic contents were analysed in all extracts obtained from roots and leaves of *S. longipedunculata* (Table I). The ethyl acetate extracts from roots (SR3) and leaves (SF3) contained the largest amounts of polyphenols with close values (147.52 and 145.32 mg/g, respectively). The concentration of polyphenols in the different roots extracts was influenced by the solvent and varied in the following order: SR3-AcOEt (147.52 mg/g) > SR1-E (76.75 mg/g) > SR4-W (45.09 mg/g) > SR2-PE (20.61 mg/g). Previously, it was

shown that the water-methanol extracts from roots of the Nigerian species contained smaller amounts of polyphenols [13]. Regarding the polyphenolic content of the leaves (Table I), the EtOAc extract (SF3) was the richest (145.32 mg/g), followed in order by the extracts: SF2 - PE (56.71 mg/g), SF1 - E (41.20 mg/g), SF4 - W (28.42 mg/g). Previous analyses were performed on chloroformic and methanolic extracts, revealing different values (below 30 mg/g in the chloroformic extract and 75 - 100 mg GAE/g in the methanolic extract) [9].

The antioxidant effect of the extracts was evaluated *in vitro* by the DPPH and FRAP methods. The EtOAc extracts (SR3 and SF3) showed the best scavenging ability on DPPH radical compared to the other extracts. The SF3 extract demonstrated a very good antioxidant activity, with the lowest IC_{50} value (31.95 µg/mL), comparable to that of Trolox. The SF3 and SR3 extracts also demonstrated a strong ferric ion reducing capacity (682.14 µM TE/g, and 465.09 µM TE/g, respectively), in good agreement with the polyphenolic content. Our findings seem to be supported by several previous studies [13]. Thus, *S. longipedunculata* species, especially the leaves extract in AcOEt, could be an important source of antioxidant phenolic compounds, capable to neutralize the reactive oxygen species and to protect against the damages of the oxidative stress.

Table I

Total polyphenol content and antioxidant activity of *S. longipedunculatae radix* and *folium* dry extracts

Samples	TPC (mg GAE/g dry extract)	DPPH IC_{50} (µg/mL dry extract)	FRAP (µM TE/g dry extract)
SR1 (95% ethanol)	76.75 ± 1.25	116.99 ± 5.01	288.80 ± 7.20
SR2 (PE)	20.61 ± 0.38	> 200	173.81 ± 3.19
SR 3 (EtOAc)	147.52 ± 2.47	60.12 ± 2.87	465.09 ± 8.91
SR 4 (water)	45.09 ± 0.40	> 200	88.47 ± 1.53
SF1 (95% ethanol)	41.20 ± 0.39	119.27 ± 2.72	154.35 ± 5.65
SF 2 (PE)	56.71 ± 1.28	> 200	101.08 ± 3.92
SF 3 (EtOAc)	145.32 ± 2.67	31.95 ± 1.04	682.14 ± 17.86
SF 4 (water)	28.42 ± 0.57	163.75 ± 2.24	96.35 ± 2.65
Trolox	-	11.20 ± 0.09	-

GAE: gallic acid equivalents; TE: Trolox equivalents.

Nitrite can induce the auto-oxidation of Hb according to the mechanism previously described [8] and the presence of antioxidants can inhibit this reaction. Figure 1 illustrates the inhibition of oxidation of Hb by nitrite in the presence of the best-performing extracts from roots (RS3) and leaves (SF3) of *S. longipendunculata*. Both extracts have a good capacity to inhibit the oxidation of Hb by nitrite, twice as much in the roots extract compared to the leaves extract. This ratio of reactivity matches the one seen in DPPH assay measurements (Table I) but not in total phenolic content (equal values between the two extracts) or FRAP (reverse order).

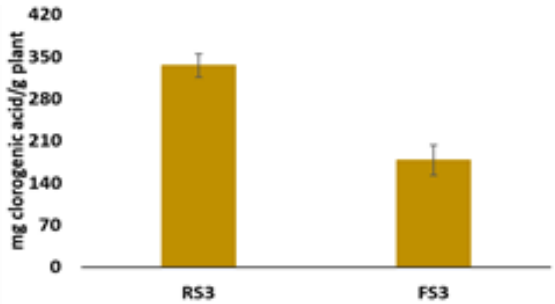

Figure 1.

The inhibition of the oxidation of haemoglobin by nitrite in the presence of *S. longipendunculata* extracts. The inflection time (t_i) is converted in mg CAE/g plant (Hb – 20 µM; nitrite – 166 µM; phosphate pH 7)

Inhibition of lipid peroxidation catalyzed by cytochrome C. Both the SR3 and SF3 extracts (from roots and leaves) inhibit the lipid peroxidation catalysed by cytochrome C at very low concentration (1.66 µg/ml). In this experiment, the antioxidant capacity is directly correlated with the length of the lag phase. The RS3 shows the longest lag phase, suggesting the best anti-oxidant capacity - in good agreement with the data from the nitrite-induced auto-oxidation (Figure 2).

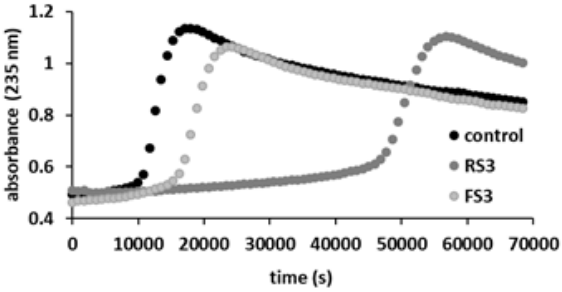

Figure 2.

Time course for the oxidation of liposomes catalysed by cytochrome C in the presence of the *S. longipedunculata* extracts (cytochrome C – 2 µM; liposomes – 0.5 g/mL; extracts – 1.66 µg/mL; phosphate – 10 mM; pH 7)

HPLC-MS analysis

The identification and quantification of flavonoids and phenolic acids from leaves and roots of *S. longipedunculata* were performed by the HPLC-MS

methods (Tables II and III). The eight different extracts were analysed using the same chromatographic conditions as those for standard substances. Identification was based on their retention times and UV and MS spectra as compared to standards. Quantification was performed using an external standard method, with 27 standard phenolic compounds [2, 4, 7].

Table II

Phenolic compounds identified by the HPLC method in the *S. longipedunculata* roots extracts

Phenolic compounds	Retention time, min	Concentrations (mg/g dry extract)			
		SR1-E	SR2-PE	SR3-AcOEt	SR4-W
Protocatechuic acid	2.8 ± 0.06	0.04 ± 0.01	-	0.14 ± 0.04	-
Gentisic acid	3.69 ± 0.04	-	-	< 0.02	-
Chlorogenic acid	6.43 ± 0.05	< 0.02	-	< 0.02	< 0.02
Vanillic acid	6.7 ± 0.06	0.46 ± 0.03	-	2.09 ± 0.10	-
Syringic acid	8.4 ± 0.08	0.32 ± 0.06	-	1.28 ± 0.06	-
Ferulic acid	12.8 ± 0.10	< 0.02	-	0.07 ± 0.01	-
Rutin	20.76 ± 0.15	-	-	-	0.03 ± 0.02

Table III

Phenolic compounds identified by the HPLC method in the *S. longipedunculata* leaves extracts

Phenolic compounds	Retention time, min	Concentrations (mg/g dry extract)			
		SF1-E	SF2-PE	SF3-AcOEt	SF4-W
Protocatechuic acid	2.8 ± 0.06	0.04 ± 0.01	-	0.54 ± 0.15	-
Gentisic acid	3.69 ± 0.04	-	-	-	-
Caffeic acid	5.60 ± 0.04	0.07 ± 0.02	-	< 0.02	-
Chlorogenic acid	6.43 ± 0.05	-	-	0.56 ± 0.13	-
Vanillic acid	6.7 ± 0.06	-	-	0.16 ± 0.02	-
Syringic acid	8.4 ± 0.08	-	-	0.07 ± 0.01	-
p-Coumaric acid	9.48 ± 0.08	0.47 ± 0.02	0.15 ± 0.03	3.62 ± 0.27	-
Ferulic acid	12.8 ± 0.10	0.21 ± 0.07	0.12 ± 0.03	1.38 ± 0.11	-
Hyperoside	18.60 ± 0.12	3.36 ± 0.23	0.54 ± 0.15	18.28 ± 1.72	< 0.02
Isoquercitrin	20.29 ± 0.10	1.68 ± 0.11	0.33 ± 0.06	16.10 ± 1.39	< 0.02
Rutin	20.76 ± 0.15	2.74 ± 0.15	0.23 ± 0.02	4.92 ± 1.07	3.41 ± 0.13
Quercitrin	23.64 ± 0.13	-	-	< 0.02	-
Quercetin	27.55 ± 0.15	0.20 ± 0.09	0.16 ± 0.02	0.79 ± 0.07	-

A total of seven compounds were found in the extracts of *S. longipedunculata* roots (Table II). The EtOAc (SR3) and ethanolic (SR1) extracts were found to have a large amount of phenolic principles compared to other extracts. Vanillic, syringic and protocatechuic acids were determined for the first time, and the EtOAc was the most appropriate solvent for their extraction. Other authors reported the presence of some polyphenolic compounds in *S. longipedunculata*, such as: chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin, quercetin [13]. Regarding the analysis of the four leaves extracts, 12 polyphenolic compounds were determined for the first time (Table III). The richest was the EtOAc extract (SF3), followed by the ethanolic extract (SF1). The SF3 extract contained large amounts of flavonoids: hyperoside (18.28 mg/g), isoquercitrin (16.10 mg/g) and rutin (4.92 mg/g), as well as phenolic acids: *p*-coumaric acid (3.62 mg/g) and ferulic acid (1.38 mg/g). The solid-liquid and liquid-liquid extraction methods are the most commonly used for the polyphenols analysis and the yield and rate of polyphenolic extraction depends on the solvent characteristics. Various polarities of solvents are able to extract various combinations of phenolic compounds. Obtaining new fractions from the initial extract, using different solvent, emphasized the presence of new compounds, which were not in the limit of detection in the initial extract. The fractions are more concentrated in those compounds, so they could be identified by the used HPLC method. Thus, the EtOAc extracts of *S. longipedunculata* leaves and roots were the richest in flavonoids and phenolic acids, which could be the main bioactive compounds responsible for the very good antioxidant capacity of these extracts.

Conclusions

In the present study, different extracts (in ethanol 95%, petroleum ether, ethyl acetate and water) from roots and leaves of *S. longipedunculata* were studied in terms of polyphenolic compounds and antioxidant activity. Our results highlight that ethyl acetate extracts from leaves showed very good antioxidant properties as well as high content of polyphenolic compounds, of which some flavonoids: hyperoside, isoquercitrin and rutin, as well as *p*-coumaric acid and ferulic acid were identified. These findings support some of the many traditional uses of this African medicinal plant, based on the antioxidant capacity of the polyphenolic compounds of the leaves and, in addition,

this raw material could be considered a potential source of new drugs.

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Conflict of interest

The authors declare no conflict of interest.

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