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Flavonoid Compounds from *Zanthoxylum leprieurii* Guill. et Perr (Rutaceae) Extracts and their Antioxidant Activity against ABTS$^{\bullet+}$

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ABSTRACT

Screening of flavonoid compounds in fruit, leaf, stem and root bark extracts of *Z. leprieurii* was achieved by liquid chromatography (LC) coupled with tandem mass spectrometry (MS^2). Among the 186 reference compounds tested, four flavonoids were identified in one or other of the organ extracts from each plant (fruit, leaf, root barks and stem): neodiosmin and hesperidin were identified in all extracts. The presence of datiscin was reported only in leaves while that of rutin was identified in fruits and leaves. Of these four flavonoids, three (neodiosmin, datiscin and rutin) are detected for the first time in *Z. leprieurii*. The antioxidant properties of different extracts were tested regarding their scavenging activities on ABTS$^{\bullet+}$ radical. Fruit, leaf and stem extracts had low antioxidant potential and root bark extracts exhibited very low antioxidant activity.

Keywords: *Zanthoxylum leprieurii*, flavonoids, LC-MS/MS and antioxidant activity.

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INTRODUCTION

Phenolic compounds, such as phenolic acids and flavonoids are widely distributed in nature and are the most abundant antioxidants in the diet; they are the common components of fruits, vegetables, and its derivatives. The biological effects derived from phenolic compounds have been attributed to their antioxidant activity. The importance of the antioxidant constituents of plant materials for health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects. Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oil seeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs [1–4].

Belonging to the Rutaceae family, *Zanthoxylum leprieurii* is a deciduous aromatic tree distributed in Central and West Africa [5]. It is used in herbal medicine for the treatment of malaria, urinary infections, rheumatic pain, skin infections, intestinal parasites, sickle cell anemia, stomach disorders and dysentery [5–7]. In Cameroon, the dried fruits are traditionally used as a spice in soups [8]. Literature has also shown that extracts of this plant possess a potential antimicrobial [9–13], insecticidal [14], antiplasmodial [15], cytotoxic [16,17], anti-inflammatory [18] and antioxidant activities [18–20].

Like many species of the Rutaceae family, flavonoids are mainly represented in the genus *Zanthoxylum* by flavones, flavonols and flavanones, which can be glycosylated and / or polymethoxylated [4,21]. Only one study have been carried out on the chemical composition of *Z. leprieurii* flavonoids [5], while the number of articles dealing with this subject is relatively important for other species of the *Zanthoxylum* genus. Other phytochemical studies on *Z. leprieurii* revealed the presence of diterpenes [22], alkaloids [15,16,23–27], amides [27] and coumarins [11,15,27] in solvent extracts from various organs (root, stem, leaf and fruit). Moreover, some papers were reported the chemical composition and biological activities of fruit essential oils [9–11,18,28–32] from various geographical origins such as Nigeria and Cameroun. Furthermore, its antioxidant activity has been

described in two works. Bouba et *al*. (2010) have been reported good antioxidant activity of fruit extracts from *Z. leprieurii* [19]. Womeni et *al*. (2013) have shown that fruit extracts from *Z. leprieurii* considerably inhibits the oxidation of crude soybean oil [20].

Therefore, the present study was designed to qualitative determination of flavonoids in fruit, leaf, stem and root bark extracts of *Z. leprieurii* from Senegal using liquid chromatography (LC) coupled with tandem mass spectrometry (MS^2). The antioxidant activities of these extracts were also determined using $ABTS^{\bullet+}$ method.

MATERIAL AND METHODS

Solvents

Methanol (HPLC grade) and hexane (HPLC grade) used for sample extraction, were purchased from Fisher Scientific (Illkirch, France). The solvents used for liquid chromatography were LC-MS grade acetonitrile (ACN), obtained from Fisher Scientific. Deionized water was purified using a Milli-Q water (Millipore, Bedford, MA, USA) purification system. Formic acid (HPLC grade) used for buffering was also purchased from Fisher Scientific.

Plant material

The fruit, leaf, stem and root bark samples of *Z. leprieurii* were harvested in November 2015 (fruit ripening period) from a single tree, growing wild in the Senegalese locality known as Colomba-Bignona (12°46′ N, 16°14′ W). The botanical identification of the plant material was performed by Dr. William Diatta from the Department of botanical and pharmacognosy of University Cheikh Anta Diop of Dakar (Senegal).

Plant extracts

Each plant organ (fruits, leaves, root barks, stems, and trunk barks) has been extracted separately. Plant samples were air dried for a period of four weeks at ambient temperature. The plant material was crushed with an average particle size of 0.2 mm using a blade miller (Polymix PX-MFC 90D, KINEMATICA AG, Luzern, Switzerland). 50 g of powder samples were extracted with 3 × 200 mL of methanol over 48 h each time, at room temperature under magnetic stirring. The solutions were combined, filtered through filter paper (PRATDUMAS, Couze-St-Front, France) and evaporated to dryness using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany). The methanolic solutions were evaporated to dryness using a rotary evaporator and the extract yields (w/w, calculated on a dry weight plant) were 42.2%, 18.7%, 18% and 8% for fruits, leaves, root barks and stems, respectively. Each dried extract was stored at 4 °C until analysis. Prior to LC-MS^2 analysis, 10 mg of each sample extract was dissolved in H_2O/ACN (1:1 v/v) to obtain a solution at a final concentration of 100 mg/L. Finally, the solutions were filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter (Whatman, Maidstone, UK).

References compounds and preparation of standard solutions

All references of flavonoids (98% purity determined by HPLC) were purchased from Extrasynthese (Geney, France). Solutions of each standard were prepared by dissolving the reference compound in ACN/H_2O (1:1 v/v) at a final concentration of 5 mg/L. Then, they were filtered with a 0.2 µm PTFE filter. These standard solutions were diluted with ACN/H_2O (1:1 v/v) to obtain calibration curves with seven points in the concentration range of 0.01–5 mg/L. The calibration solutions were stored at 4 °C until LC-MS^2 analysis. A blending solution, which contained the reference components at a concentration of 0.1 mg/L in H_2O/ACN (1:1 v/v), was used as positive control of LC-MS^2 analysis of the plant extracts (before and after sample injections).

MS^2 Conditions

MS^2 conditions were carried on an AB Sciex (Toronto, ON, Canada) 3200 QTRAP linear triple quadrupole fitted with electrospray ionization (ESI) ion source operating in negative mode. High purity nitrogen was used as both a nebulizer and turbo gas. The ESI source was operated with following settings in negative mode; curtain gas: (CUR) 25 psi, nebulizer gas: (GS1) 41 psi, heater gas: (GS2) 65 psi, ion spray voltage (IS): -4200 V, and temperature: 550°C. Standard solutions (component concentration: 0.1 mg/L) were directly infused at the flow rate of 10 µL/min in the MS/MS apparatus. Multiple EPI mass spectra of each compound were recorded in the range of m/z = 50–1000 at 4000 Da/s. IDA properties were set to select 1 to 2 peaks above 500 counts with an exclusion filter after 5 occurrences for 30 s with dynamic background subtraction. The software used for data acquisition and data analysis was Analyst 1.5.2 (AB Sciex, Framingham, MA, USA).

LC conditions

The LC system consisted of a Flexar LC Perkin-Elmer (Waltham, MA, USA) with two Flexar FX-10 LC pumps, a Flexar solvent manager, a 275-Flexar autosampler, and a Flexar LC PE200 column oven. LC analyses were performed on a 100 mm × 2.1 mm i.d. 3 µm, LUNA 3U C18 column (Phenomenex, Torrance, CA, USA) and the column temperature was set at 25 °C. A volume of 10 µL of sample was injected using an injection loofcp of 15 µL in partial loop mode. The mobile phase consisted of MilliQ water containing 0.1% formic acid (solvent A) and ACN (solvent B). The flow rate was set at 500 µL/min. The column was equilibrated (A:B; v/v) in 90:10 (5 min), and elution was carried out with the following steps; 90:10 (2 min), a linear gradient increasing from 10% B to 100% (14 min), and 100% B (9 min).

Identification of the components

To detect 186 components potentially present in the Rutaceae family (commercially available standards) in the studied extracts, we used the method based on LC-MS^2 developed in previous work [4]. The identification of flavonoids in *Z. leprieurii* extracts was allowed by comparing the retention times, observing the characteristic MRM transitions, and by matching the MS^2 spectra of reference compounds.

For each reference compound, a relevant transition of the precursor-to-product ions was detected with the utilization of the multiple reaction monitoring (MRM) mode. Using ESI source operating in negative mode, the precursor ion $[M + H]^-$ for each of the analytes was determined in MS^1 full scan tests and the product ions in MS/MS experiments. MRM transitions of each compound were optimized using direct infusion with the following MS/MS parameters: declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE), and collision cell exit potential (CXP). Retention times of reference compounds were determined by LC-MS^2 analysis in the multiple reaction monitoring (MRM). Mass spectra of standard components were performed by the MRM mode followed by an enhanced product ion (EPI) scan, triggered by information dependent acquisition (IDA) criteria.

Antioxydant activity. ABTS$^{•+}$-Scavenging Assay

The ability of extracts to bleach the 2,2'-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) radical (ABTS$^{•+}$) was evaluated according to the method of modified Andreani *et al* (2013) [33]. ABTS$^{•+}$ was produced by reaction of equal volumes of 7 mM ABTS solution and 2.4 mM potassium persulfate for 16 h, in the dark and at room temperature. The stock solution was then diluted in Milli-Q water to reach an absorbance of 1.00 ± 0.01 at 734 nm. Then, 200 µL of diluted ABTS$^{•+}$ solution was mixed with 50 µL of methanol extract solution (0 – 200 µg/mL final concentration). The absorbance at 734 nm was taken 5 min after mixing using a Tecan Infinite® 200 PRO multimode reader (Tecan Group Ltd., Männedorf, Switzerland). Radical scavenging activity was calculated using the following formula:

$$I\ \%=100 \times (Ab-As)/Ab$$

where *Ab* is the absorbance of the control reaction and *As* the absorbance of the sample. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against relatively to the sample concentration. Tests were carried out in triplicate, and Trolox was used as a positive control.

RESULTS AND DISCUSSION

Analysis of flavonoid compounds from *Z. leprieurii* extracts using the LC-MS^2 Method

The identification of flavonoids in *Z. leprierii* extracts was allowed by the comparison of retention times, the observation of characteristic MRM transitions, and by matching the MS^2 spectra of reference compounds (Table 1). The mobile phase H_2O/ACN (1:1 v/v), allowed the separation of targeted compounds of *Z. leprieurii* extracts.

Table 1: Retention times (Tr), multiple reaction monitoring (MRM) transition, and optimized tandem mass spectrometry (MS/MS) detection parameters of four flavonoids.

No.	Compounds	Tr (min)	Transition		MS Parameters (V)				
			Q1 Mass (Da)	Q3 Mass (Da)	[a]DP	[b]EP	[c]CEP	[d]CE	[e]CXP
1	Neodiosmin	8.29	607	299	−90	−4	−28	−36	−4
2	Datiscin	8.34	593	285	−95	−6.5	−34.1	−50	−4
3	Rutin	8.46	609	301	−85	−9.5	−26	−36	−4
4	Hesperidin	8.48	609	301	−75	−4.5	−32	−34	−4

[a]DP = declustering potential; [b]EP = entrance potential; [c]CEP = collision cell entrance potential; [d]CE = collision energy; [e]CXP = collision cell exit potential.

Among the 186 reference compounds tested, four flavonoids were unambiguously identified in one or other of the organ extracts from each plant (fruit, leaf, root barks and stem). These four compounds (table 2) are: one flavone (neodiosmin **1**), two flavonols (datiscin **2,** rutin **3**) and one flavanone (hesperidin **4**).

The flavonoid content of *Z. leprieurii* extracts showed a variation in the chemical composition according to the parts of the plant. Two of these flavonoids (neodiosmin **1** and hesperidin **4**) were present in stems and roots, three (neodiosmin **1**, rutin **3** and hesperidin **4**) in fruits and all four were detected in the leaves. Thus, two glycosylated flavonoids (neodiosmin **1**, hesperidin **4**) were identified in all extracts. The presence of rutin **3** was recognized in fruits and leaves while that of datiscin **2** was reported only in leaves.

Table 2: Identified flavonoids in various parts of *Z. leprieurii* plant

No.	Compounds	Fruits	Leaves	Stems	Root barks
1	Neodiosmin	+	+	+	+
2	Datiscin	−	+	−	−
3	Rutin	+	+	−	−
4	Hesperidin	+	+	+	+

(+): present; (-): absent

Of these four flavonoids, three are detected for the first time in *Z. leprieurii*, namely neodiosmin, datiscin and rutin. The presence of hesperidin in Z. *leprieurii* was reported by Tabuti in 2011 [5]. All the flavonoids identified in this study have already been reported in Rutaceae, particularly in citrus fruits; thus, these constituents can be considered as chemotaxonomic markers of the genus *Zanthoxylum*.

Antioxydant activity

The antioxidant properties of all extracts were tested regarding their scavenging activities on ABTS$^{•+}$ radical (Table 3). The ABTS$^{•+}$ scavenging activity of extracts decreased in the order of fruit extract > leaf extract > stem extract > root extract. Compared to the reference antioxidant (Trolox: 4.18 µg/mL), fruit, leaf and stem extracts had low antioxidant potential with an IC_{50} of 51.41±0.94 µg/mL, 55.10±2.14 µg/mL and 60.66±4.85 µg/mL, respectively. Extract obtained from root barks exhibited very low antioxidant activity (IC_{50} 95.40±2.27 µg/mL). Our results cannot be compared to those of the literature because the methods used are not the same.

Table 3: ABTS$^{•+}$-Scavenging Activity

Samples	IC_{50} [µg/mL]
Leaves	55.10±2.14
Fruits	51.41±0.94
Root barks	95.40±2.27
Stems	60.66±4.85
Trolox	4.18±0.05

CONCLUSION

This study reported flavonoid compounds from *Zanthoxylum leprieurii* and their antioxidant activity. Four flavonoids (neodiosmin, datiscin, rutin and hesperidin) were identified. The different extracts had low antioxidant potential. In perspective, we will quantify these falvonoids.

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