Characterization of Leaf Phenolic Compounds of Sabicea johnstonii by HPLC-MS⁺

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ABSTRACT

Sabicea johnstonii is reported among wild plant species used by Congolese people for certain purposes. The leaves of this plant are used for beverages. They are also used to lower blood pressure and to treat burns. In a recent study in vitro, it was shown that the scavenging capacity and the reducing power of the leaves decoction of S. johnstonii were mainly due to their polyphenols. They can be used to treat some health problems. The objective of this research work was to characterize the major phenolic compounds of these leaves by HPLC-MS⁺. Fresh leaves of S. johnstonii, collected at Masako, were drying and their phenols were extracted with methanol for their characterization by HPLC-MS⁺. These analyses showed the presence of ten major polyphenolic compounds: dicaffeoylquinic acid, three procyanidins, two quercetin-0-glycosides, two kaempferol-0-glycosides and two unidentified phenols. The presence of these compounds and the observed antioxidant activities in another study justify the use of these leaves in Congolese traditional medicine and as beverage.

1. Introduction

Plants containing polyphenolic compounds are sometimes used to treat many health problems. Epidemiological and experimental studies showed that these polyphenols play a role in preventing various diseases including cancer, diabetes and cardiovascular or neurodegenerative diseases [1, 2]. They can destroy free radicals and reduce the risk of these diseases. They can be used to treat other health problems such as to lower blood pressure and to treat burns. Sabicea johnstonii (Rubiaceae) [3] is a spontaneous liana. Leaves and corolla of this plant are red-carmine and blackish (dry state) respectively. These leaves are consumed as tea by Kumu people of Masako (Forest reserve, DR Congo) [4]. They are sometimes used to lower blood pressure. The Lega people (DR Congo) used the powdered dry leaves of this plant to treat burns [5]. In the Ituri forest, Mbuti and Efe hunter-gatherers use it as a magic plant [6].

A recent study [7] on some wild plant teas collected at Masako showed that the leaf decoction of S. johnstonii contains saponins and polyphenols including flavonoids. It does not contain alkaloids, cyanides and oxalates. This study showed also that these polyphenols are responsible for the scavenging capacity and the reducing power observed.

It is known that HPLC-MS⁺ is a powerful tool for the analysis of natural substances. The purpose of the present research was to characterize by HPLC-MS⁺ the major phenolic compounds of the methanolic leaf extract of S. johnstonii.

2. Experimental Methods

2.1 Plant Material

Fresh leaves of S. johnstonii were collected in the forest reserve of Masako and were identified at the Faculty of Sciences (University of Kisangani, DR Congo). After drying in darkness at room temperature for five days in the Chemistry laboratory of this faculty, the leaf powder was packed for analysis in the “Polyphenols BIOTECH” laboratory (France).

2.2 HPLC-MS⁺ Analysis

HPLC is a separation method of choice for natural substances analysis. Mass spectrometry provides information about the molecular mass and the fragmentation patterns of a compound. The HPLC-MS⁺ system used for analysis consisted of a HPLC system Agilent technologies (Automatic injector and Diode-array detector 1200 series, ProntoSil column type C18: 4 mm x 250 mm, 5 μm) and a Mass spectrometer Esquire 6000 (Bruker Daltonics Bremen) equipped with an electrospray ionization source.

Phenolic compounds of the leaf powder were extracted with methanol. Their separation was performed at 25 °C with solvents A (Water/formic acid 0.1%) and B (Acetonitrile/ Formic acid 0.1%). The injection volume and the elution flow rate were 20 μL and 21.6 mL/min respectively. The elution program was used as shown in Table 1.

Table 1 Elution pattern of the extract

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>2</th>
<th>15</th>
<th>40</th>
<th>44</th>
<th>45</th>
<th>50</th>
<th>51</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Solvent B</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>35</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td>1</td>
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The detection of phenolic compounds was carried out at 280 nm. Mass spectra were acquired using electrospray ionization in positive or negative mode. They were obtained in full scan MS mode from 150 to 1200. ESI-MS parameters were as follows: potential of the ESI source, 4 kV; capillary temperature, 350 °C; nebulizer, 35 psi; dry gas, 10/l/min. Data were collected and treated with Hystar logical version 3.0. The MS⁺ fragmentations of these compounds and the literature data were used for their identification.

3. Results and Discussion

HPLC-DAD-MS was used for characterizing polyphenolic compounds of the methanolic leaf extract of S. johnstonii. The Chromatogram of the HPLC separation is shown in Fig. 1. The analysis of this figure showed that leaves of S. johnstonii contain ten major polyphenolic compounds listed in Table 2.

Some of these compounds have a high molecular weight, suggesting they belong to the class of complex polyphenolic compounds. MS⁺ fragmentations (positive or negative mode) of these compounds were used for obtaining more information on their structures. The results of these fragmentations are presented in Table 3.
Procyanidin consis
ting of monomers ranging from dimers to polymers. The
st of monomers ranging from dimers to polymers. The
}-ions were also observed after elimination
-elimination of the moiety from the ion of m/z 867. On this mode, the ions of m/z 731, 715 and 663 lost 152 Da, 306 Da (or 138 Da) and 308 Da (loss of gallic acid 170 Da plus a BFF fragment 138 Da) giving rise to product ions of m/z 579, 409 (or 775) and 355 respectively.

The anions of m/z 847, 695 and 577 lost 152 Da [elimination of galloyl unit] giving rise to product ions of m/z 695, 543 and 425 respectively.

The MS 
fragmentation resembled to the fragmentation of a

These fragmentations produced losses and fragment ions characteristic of trimeric procyanidins. Based on the above data, compound 5 was recognized as a procyanidin dimer (C_{16}H_{20}O_{10}) [13].

3.3 Compound 3 (MW 748 Da)

MS 
and MS -fragments of compound 3 on positive or negative mode did not provide sufficient structural information of this compound.

3.4 Compound 4 (MW 1154 Da)

MS 
fragmentation of compound 4 (m/z 1153) gave [M+H]- ion at m/z 867 [interflavonic bond cleavage] and [M+H]- ion at m/z 579 [two interflavonic bond cleavages].

MS 
fragmentation of this compound gave [M+H]- ion at m/z 577 (loss of a dimer) and an ion at m/z 291 corresponding to (epi)catechin.

MS 
fragmentation of compound 4 (m/z 1153) gave [M+H]- ion at m/z 865 [interflavonic bond cleavage] and [M+H]- ion at m/z 576.

MS 
fragmentation of this compound gave [M+H-203] ion at m/z 1065, [M+H-306] ion at m/z 847 (loss of a RDA fragment with 168 Da plus a benzofuran-forming "BFF" fragment with 138 Da), [M+H]- ion at m/z 739 [interflavonic bond cleavage plus the loss of an HRF fragment with 126 Da] and [M+H]- at m/z 577 [two interflavonic bond cleavages].

Procyanidin consist of monomers ranging from dimers to polymers. The presence of (epi) catechin (290 Da) as monomer was also observed on positive mode (MS+ at m/z 663 [535-(epi) catechin] and at m/z 287 on negative mode [577-(epi) catechin].

On positive mode, the loss of 288 Da was also observed after elimination of the moiety from the ion of m/z 867. On this mode, the ions of m/z 731, 715 and 663 lost 152 Da, 306 Da (or 138 Da) and 308 Da (loss of gallic acid 170 Da plus a BFF fragment 138 Da) giving rise to product ions of m/z 579, 409 (or 775) and 355 respectively.

The MS 
fragmentation resembled to the fragmentation of a
procyanidin tetramer analyzed by [12] (m/z: 1153 [M]+), MS 983, 865, 695, 577.

These fragmentations produced losses and fragment ions characteristic of trimeric procyanidins. Based on the above data, compound 4 was characterized as a procyanidin tetramer (C_{16}H_{20}O_{10}) [13].

3.5 Compound 5 (MW 756Da)

MS 
and MS -fragments of compound 5 on positive mode gave a [M+H-Deoxyhexosyl] ion at m/z 611, [M+H-2Deoxyhexosyl] ion at m/z 645 and [M+H-2Deoxyhexosyl-Hexosyl] ion at m/z 735, 303, corresponding to quercetin (aglycone).

The presence of quercetin (302 Da) as aglycone was also observed on negative mode (MS- at m/z 367 [669-Quercetin] and at m/z 300 (from homolytic cleavage, [14]).

MS 
fragmentation of this compound on positive mode gave an ion at m/z 611, after losing 146 Da (deoxyhexosyl unit), suggesting its attachment on quercetin skeleton. The cleavage of 308 Da from the m/z 611 ion gave a protonated quercetin, what suggested also the attachment of a deoxyhexosylhexose or coumaroylhexose on quercetin skeleton.

These fragmentations were typical of flavonoid O-glycosides. Based on these data, compound 5 was a quercetin O-glycoside, its structure might
be either quercetin 0-deoxyxoside-0-deoxyxosyhexoside or quercetin O-deoxyxoside-O-coumaroylhexoside. This compound is an isomer of quercetin 3-0-rutinoside-7-O-rhamnoside and quercetin 3-O-deoxyhexosyhexoside 7-O-deoxyxoside analyzed previously by few workers [10, 15].

3.6 Compound 6 (MW 740 Da)

MS/fragmentation of compound 6 on positive mode produced [M+H-Deoxyhexosyl-] ion at m/z 741 and [M+H-2Deoxyhexosyl-Hexosyl-] ion at m/z 727, corresponding to kaempferol (aglycone).

On the same mode, MS/fragmentation of this compound gave [M+H-H2O] at m/z 723, [M+H-Deoxyhexosyl-] ion at m/z 595, [M+H-2Deoxyhexosyl-Hexosyl-] ion at m/z 449 and [M+H-2Deoxyhexosyl-Hexosyl-] ion at m/z 287 (aglycone).

The presence of kaempferol as aglycone was also observed on negative mode (MS') at m/z 146 Da on quercetin skeleton. The cleavage of a deoxyhexosylhexoside or coumaroylhexoside moiety with the fragmentation of caffeoylquinic acid ion at m/z 191 [M+H-Caffeoyl]2+ at m/z 353 [M+H-Caffeoyl]2+ ion at m/z 209[Caffeic acid] at m/z 163 [M+H-Caffeoyl]2+ ion at m/z 146 [M+H-Caffeoyl]2+ ion at m/z 595, [M+H-Deoxyhexosyl-Hexosyl-] ion at m/z 449 and [M+H-2Deoxyhexosyl-Hexosyl-] ion at m/z 287 (aglycone).

3.7 Compound 7 (MW 516 Da)

MS/fragmentation of compound 6 generated [M+H2O]+ ion at m/z 499 [M+H-H2O]1+ ion at m/z 163, which corresponds to caffeoyl unit.

MS/fragmentation of this compound produced a loss of caffeoyl quinic acid 146 Da on quercetin skeleton. The cleavage of a deoxyhexosylhexoside or coumaroylhexoside moiety with the fragmentation of caffeoylquinic acid ion at m/z 353 [M+H-Caffeoyl], which corresponds to caffeoyl quinic acid.

MS/fragmentation of this compound gave an ion at m/z 353 [M+H-Caffeoyl], which fragmented to produce an ion at m/z 191 [M+H-Caffeoyl]2+ ion at m/z 146 [M+H-Caffeoyl]2+ ion at m/z 595, [M+H-Deoxyhexosyl-Hexosyl-] ion at m/z 449 and [M+H-2Deoxyhexosyl-Hexosyl-] ion at m/z 287 (aglycone).

These fragmentation data are in agreement with the fragmentation of hydroxycinnamates reported earlier [18, 19]. They are also in agreement with the fragmentation of caffeoylquinic acid found by Parveen et al [20].

3.8 Compound 8 (MW 902 Da)

MS/fragmentation of compound 8 produced an ion at m/z 757 [M+H-Deoxyhexosyl-] which generated an ion at m/z 601 [M+H-Quercetin-].

MS/fragmentation of this compound produced [M+H-H2O]+ ion at m/z 885, [M+H-Deoxyhexosyl] ion at m/z 757, [M+H-2Deoxyhexosyl-Hexosyl] ion at m/z 739, [M+H-2Deoxyhexosyl-Deoxyhexosyl-hexosyl] ion at m/z 303 (Quercetin).

MS/fragmentation of compound 8 produced two ions : [M-H-Deoxyhexosyl] ion at m/z 755 and [M-H-2Deoxyhexosyl-Deoxyhexosyl-hexosyl] ion at m/z 301 (Quercetin aglycone).

Like compound 6, the fragmentation patterns of compound 8 were typical of flavonoid O-glycosides. These data allow us to suggest that compound 8 is quercetin 0-deoxyxoside-0-deoxyxosyhexoside or quercetin O-deoxyxoside-0-deoxyxosyhexoside or quercetin O-deoxyxoside-0-deoxyxosyhexoside-O-coumaroylhexoside (C6H8O18). This compound (MS') at m/z 901 [M+H]- 755, 301 is the isomer of two unidentified conjugated quercetin (MS') at m/z 901 [M+H]- 755, 609, 301 and 901 [M+H]- 755, 609, 463, 301 present in tea and analyzed by Del Rio et al [21].

3.9 Compound 9 (MW 886 Da)

MS/fragmentation of compound 9 produced only three fragments as ions: [M+H-Deoxyhexosyl]+ ion at m/z 741, [M+H-2Deoxyhexosyl]+ ion at m/z 595 and [M+H-2Deoxyhexosyl-Deoxyhexosyl-hexosyl] ion at m/z 287 (Kaempferol aglycone).

These fragmentation data are in agreement with the fragmentation of compound 9 on positive mode produced the glycosylation of kaempferol with a deoxyhexosylhexoside and a deoxyhexosylexosose or coumaroylhexose.

These fragmentation data are similar to the spectrum of flavonoids with the same molecular weight as the compound 9, his MS/fragmentations on positive or negative mode did not provide sufficient information about its structure.

4. Conclusion

Leaves of S. johnstonii contain ten major phenolic compounds: dicaffeoylquinic acid, three procyandin B, four flavonois-0-glycosides (two quercetin and two kaempferol derivatives) and two unidentified phenols.

These leaves are another source of natural antioxidants which may have positive effects on certain diseases and burns. This justifies their use in treating some health problems.

References


C.M. Bawa et al. / Journal of Natural Products and Resources 2(2) (2016) 86–89

89


