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Short Communication

High-performance liquid chromatographic determination of the major saponin from *Opilia celtidifolia* Guill. Perr.

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method for the identification of four bidesmosidic saponins of *Opilia celtidifolia* Guill. Perr. leaves is produced. The determination of the major saponin was realized with an external standard. The method was validated and two samples of leaves were analysed.

INTRODUCTION

For many years, high-performance liquid chromatography (HPLC) has been used for qualitative and quantitative analyses for the constituents of crude drugs and extracts. Many papers concerning the separation of saponins by HPLC have been reported. The reversed-phase method is the most often described and the solvent generally used is a mixture of water and acetonitrile either in the isocratic mode or with an elution gradient [1–3]. Acids (orthophosphoric acid and trifluoroacetic acid) have been added to the mobile phase to improve the separation of monodesmosidic saponins or saponins including glucuronic acid [4,5].

This paper describes the separation and determination of bidesmosidic saponins of *Opilia celtidifolia* leaves. *Opilia celtidifolia* Guill. Perr. is an African medicinal plant known for its traditional therapeutic applications. The bark is used as an anthelmintic and the leaves are employed in the treatment of oedema and dental abcesses [6–8]. Previously we reported the isolation of six saponins from barks and leaves [9]. Their structures were elucidated by mass and ^{1}H and ^{13}C NMR spectrometry (Fig. 1). Until now, no method has been described for assaying opilia raw materials. In this paper we propose the determination of the major bidesmosidic saponin (**2**) by HPLC using an external standard and identification of the other known bidesmosidic saponins (**3–6**).

EXPERIMENTAL

Plant material

Leaves of *Opilia celtidifolia* were collected from the Ivory Coast (Bouake) (sample 1) and Burkina Faso (sample 2).

Sample preparation

An extract of *Opilia celtidifolia* was prepared

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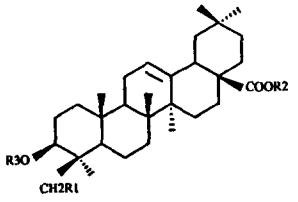


Saponins	R1	R2	R3
1	H	H	Aglu 3 — 1 Rham
2	H	Glu	"
3	OH	Glu	"
4	H	Glu	Aglu 3 — 1 Rham; 2 — 1 Glu
5	H	Glu	Aglu
6	H	Glu	Aglu 3 — 1 Rham; 2 — 1 Xyl
Arvensoside C (7)	H	Glu	Glu 2 — 1 Glu; 3 — 1 Gal

Aglu : β-D-glucuronopyranoside ; Glu : β-D-glucopyranoside ; Xyl : β-D-xylopyranoside ; Rham : α-L-rhamnopyranoside ; Gal : β-D-galactopyranoside.

Fig. 1. Structures of saponins.

by extracting 10 g of leaves with 100 ml of 80% aqueous methanol after 12 h of maceration.

Saponin standards (Fig. 1) were obtained by extraction and purification from *Opilia celtidifolia* [9]. Samples were dissolved in methanol to give a concentration of 1 mg/ml.

Apparatus and conditions

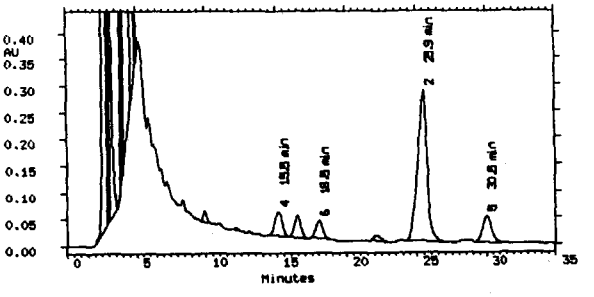
The liquid chromatograph consisted of an automatic sample injector (Waters WISP 712), two solvent-delivery systems (Waters M510) and a variable-wavelength UV detector (Waters Model 490), connected to a computer to monitor chromatographic parameters and process data.

The column was Nucleosil C_{18} (5 μm) (250 × 4.6 mm I.D.) from Interchim (Montlugon, France) and a μBondapak C_{18} guard column (10 μm), Guard-Pak insert (Waters), was also used. The eluent was acetonitrile (HPLC grade; Carlo Erba, Milan, Italy)–water (pH adjusted to 2.65 with concentrated orthophosphoric acid) (33:67, v/v). Solvents were filtered through a Millipore filter (0.45 μm). The flow-rate was 1 ml/min, the injection volume was 20 μl and detection was at 210 nm.

RESULTS AND DISCUSSION

Chromatograms of two extracts of *Opilia celtidifolia* leaves are shown in Fig. 2. Complete separation of four saponins was achieved in 35 min. All peaks were well resolved. Bidesmosidic saponins in the samples were identified by comparison with the retention times of standards (Table I). Saponin **1** is not eluted with this system. The same saponins were found in the two opilia leaf samples. Saponin **3** was not present in the leaves (Fig. 2).

Determination of saponin **2** in these extracts was achieved by the external standard method.



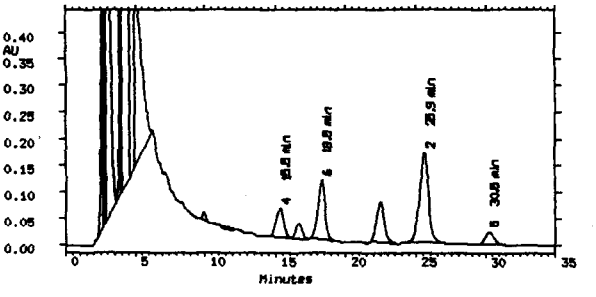


Fig. 2. Chromatograms of extracts of *Opilia celtidifolia* leaves. For identification of peaks, see Table I and Fig. 1

TABLE I

RETENTION TIMES OF SAPONINS

Saponin	t_R (min)
3	11.1
4	15.5
6	18.5
2	25.9
5	30.5

Method validation

The method was tested on saponin **2**. The linearity of the response *versus* concentration curve was investigated in the range of 0.6–3 mg/ml. Data for least-squares regression analysis of the calibration graph were $y = 2.36 \cdot 10^{-7}\ x - 1.33 \cdot 10^{-2}$ ($r = 0.9992$), where y = concentration in mg/ml, x = peak area and r = correlation coefficient.

The reproducibility of the method was calculated by assaying ten replicates of the same sample at a concentration of 1 mg/ml. For saponin **2** the relative standard deviation was estimated to be 1.17%. The reproducibility of standard preparation was tested by assaying five preparations at a concentration of 1 mg/ml. The relative standard deviation was estimated to be 0.57%. The reproducibility of the extraction method was tested for saponin **2**. Five extractions were tested. The relative standard deviation was 2.76%.

TABLE II

DETERMINATION OF SAPONIN **2** IN TWO *OPILIA CELTIDIFOLIA* LEAF SAMPLES

Sample	Saponin **2** (g per 100 g)
1 (Ivory Coast)	3.86
2 (Burkina Faso)	3.32

The detection limit was calculated to be 3 μg/ml for saponin **2** at a signal-to-noise ratio of 2:1.

These results indicate that the method is suitable for the determination of saponin **2** in *Opilia celtidifolia* leaves.

Determination of saponin 2

The determination of saponin **2** was carried out by external standardization in two opilia leaves samples. The results are given in Table II and Fig. 2.

CONCLUSIONS

The identification of bidesmosidic saponins in *Opilia celtidifolia* leaves and the determination of the major compound (saponin **2**) is possible by the proposed method.

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