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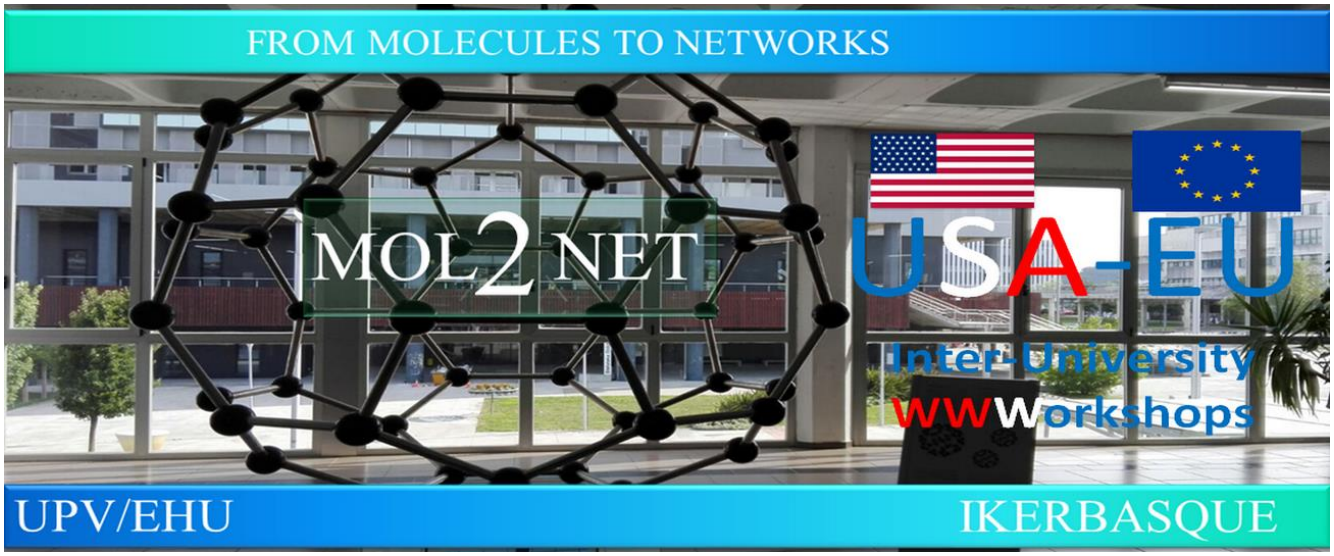

UHPLC-HRMS analysis of *Coccoloba cowellii*, an endemic endangered plant from Cuba

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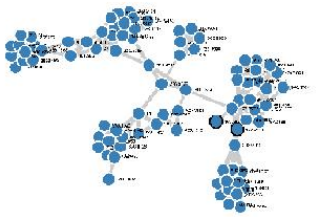
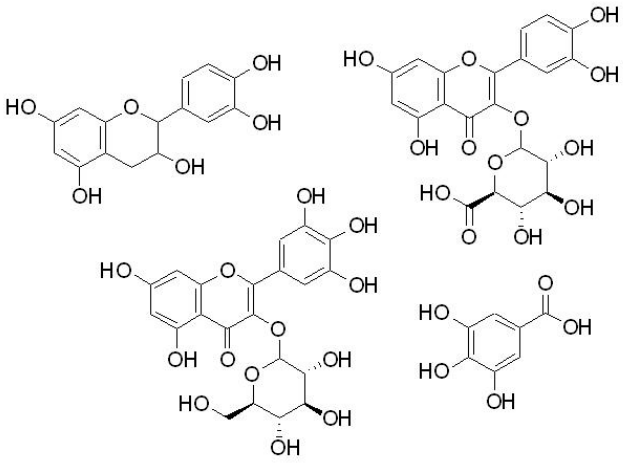
Graphical Abstract

Coccoloba cowellii



Dereplication strategy



Abstract.

Coccoloba cowellii Britton (Polygonaceae) is an endemic and critically endangered plant that only grows in Camagüey, province of Cuba. In this study a total of 13 compounds were identified in a methanolic leaf extract, employing a dereplication of the UHPLC-HRMS data by means of Feature-Based Molecular Networking (FBMN) analysis in the Global Natural Products Social Molecular (GNPS) Network, together with the results obtained from the MS-DIAL software, the interpretation of the MS/MS data and comparison with the literature. The major constituents were glucuronides and glycosides of myricetin and quercetin, as well as epichatechin-3-O-gallate, catechin, epicatechin and gallic acid, all of them being reported for the first time in *C. cowellii* leaves. The constituents identified highlight the potential of *C. cowellii* leaves, increasing the interest in the implementation of conservation strategies for this endangered species.

Introduction

Cuba is recognized as the island with the highest degree of endemism in the West-Indies, including more than half of its plant species [1]. Since plants in Cuba are frequently subjected to harsh environmental conditions (e.g., high temperature, drought, high levels of sunlight, salinity, nutrient-poor soil conditions…), combined with the fact that Cuban flora is pharmacologically and chemically under-investigated [2], the development of conservation strategies to preserve the plant species of the island is necessary.

The genus *Coccoloba* comprises approximately 120-150 species of flowering plants from the subfamily Erigonoideae of the Polygonaceae family, order Caryophyllales. It is native to the tropical and subtropical regions of America, i.e. South America, the Caribbean and Central America, with two species that extend to Florida [3]. A small number of species of *Coccoloba* are used in traditional medicine in tropical and subtropical regions of the Americas related to the treatment of several ailments [4–6]. The phytochemistry of the genus has not widely been explored, and most of the studies are centered on the more common species *C. uvifera* (sea grape, native to coastal beaches throughout tropical America and the Caribbean).

In Cuba, the presence of 34 species of *Coccoloba* has been reported [7]. From them, 25 are recognized as endemic. One of the almost unknown endemic species of this genus that grows in Cuba is *Coccoloba cowellii* Britton, which classifies as critically endangered (CR) according to the International Union for Conservation of Nature (IUCN)[8]. Ultrahigh-performance liquid chromatography – high resolution mass spectrometry (UHPLC-HRMS) was selected as analytical technique suitable for studying the non-volatile phytochemical composition of *C. cowellii* leaves, collecting as little plant material as possible.

Materials and Methods

Plant material collection and processing

Leaves of *Coccoloba cowellii* were collected near to Albaisa, in the municipality of Camagüey (Lat. 21.43615, Long. -77.83253), Cuba. The plant material was taxonomically identified by the curator of "Julián Acuña Galé" herbarium at the University of Camagüey (HIPC, http://sweetgum.nybg.org/science/ih/herbarium-details/?irn=124935), where a voucher specimen was deposited (number 12057).

The plant material (0.35 kg of fresh leaves), after cleaning, was dried at room temperature until constant weight and subsequently ground using a mill. The dried leaves (0.25 kg) were defatted with n-hexane and later on exhaustively stirring macerated in 250 mL of 80% methanol/water mixture (v/v) at room temperature during five days. Every 24 h, the solvent was collected and the material macerated with other 250 mL. The filtrate was concentrated using a rotary evaporator under reduced pressure below 40 °C. The resulting reduced filtrate was freeze dried, yielding 25.07 g dry total extract and stored at -20 °C until further use.

UHPLC-HRMS analysis

The analysis of the *Coccoloba cowellii* extract was carried out using a LC-HRMS method according to Bijttebier et al., 2016 [9] and Baldé et al., 2020 [10]. For the HPLC-DAD-QTOF analyses, accurate mass measurements were done using a Xevo G2-XS QTOF spectrometer (Waters, Milford, MA, USA) coupled with an ACQUITY LC system equipped with MassLynx version 4.1 software. Data were recorded using MS^E in the positive and negative ionization modes (two analyses per mode), and a ramp

collision energy from 10 to 30 V was applied to obtain additional structural information. Leucine-encephalin was used as the lock mass. UV detection was performed at 360 nm.

Data processing

The HPLC-MS raw data were converted to abf files (ReifycsAbf Converter) and processed with MS-DIAL version 4.24 [11]. The alignment results were exported using the GNPS export function of MS-DIAL. A molecular network was created with the Feature-Based Molecular Networking (FBMN) workflow [13] on the Global Natural Products Social (GNPS) molecular networking web-platform (https://gnps.ucsd.edu) [14]. The data were imported into Cytoscape v3.7.2 (The Cytoscape Consortium, New York, NY, USA) for visualization.

Results and Discussion

UHPLC-HRMS analysis

A qualitative analysis of the chemical composition of *C. cowellii* leaves was carried out using UHPLC-UV-QTOF-ESI-MS in negative ionization mode. Figure 1 shows the base peak intensity (BPI, peaks 1 to 15 corresponding to Table 2) chromatogram at 280 nm (*a*) and in MS negative ionization mode (*b*) of *C. cowellii* leaf extract. From the peak intensity of the UV chromatogram (Figure 1a), is inferred that compound 6 (Rt=10.60 min) appears as main compound. Peaks 7, 12 and 13 (Rt=10.85, 12.02 and 12.37) also reach high concentration ratios regarding the rest of compounds.

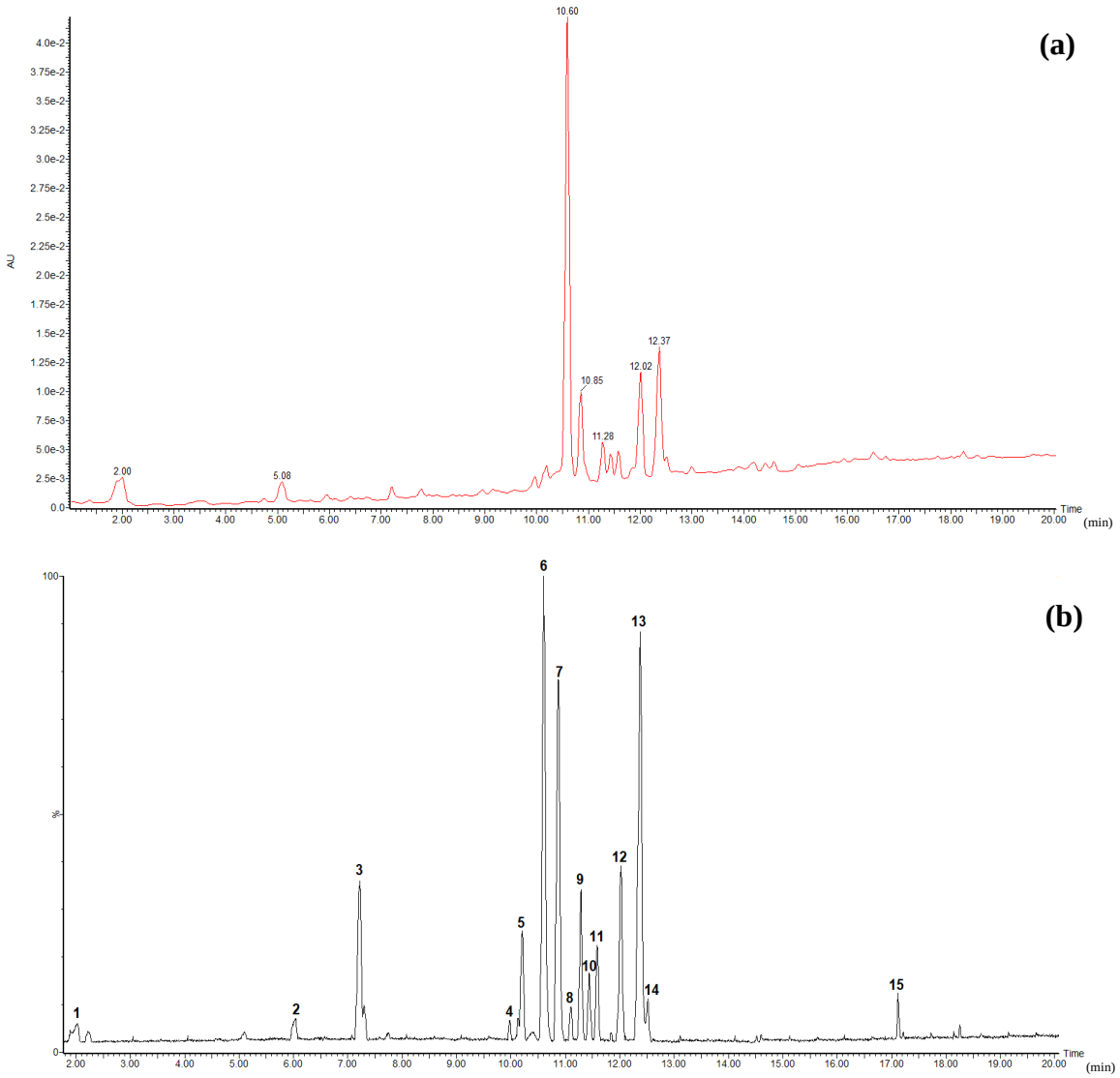


Figure 1. HPLC-DAD/QTOF-MS chromatograms of the 80% methanol extract of *C. cowellii* leaves: **(a)** UV detection at 280 nm and **(b)** base peak intensity (BPI) chromatogram (negative ion mode).

A dereplication strategy was used to analyze the raw data obtained. With this purpose, the spectra in the network were searched and matched with GNPS spectral libraries rendering 12 library hits (Table 1). The molecular networking job can be publicly accessed at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=a2f9e6e25ca64043a36a3d2fb09270c5. The matched compounds were mainly glycosides and glucuronides of the aglycones quercetin and myricetin, proanthocyanidins and one methoxylated flavonoid.

Table 1. Library hits found in the spectra of the methanolic extract of *C. cowellii* against the GNPS database.

Compound name	Library class	Cosine	Shared peaks	MZErrorPPM	LibMZ
Quercetin-3-*O*-rhamnoside (Quercitrin)	Bronze	0.85	8	1	447.093
Quercetin-3-*O*-galactoside (Hyperoside)	Bronze	0.80	7	0	463.088

Quercetin-3-*O*-arabinoside (Avicularin)	Bronze	0.72	6	0	433.078
Quercetin-3-*O*-glucuronide (Miquelianin)	Bronze	0.84	6	1	477.067
Quercetin 3-(2-galloylglucoside)	Bronze	0.73	6	37	615.099
Myricetin-3-*O*-pentoside	Bronze	0.85	6	10	449.067
Myricetin-3-*O*-galactoside	Bronze	0.93	9	2	479.083
4'-*O*-Methylmyricetin-3-*O*-rhamnoside (Mearnsitrin)	Gold	0.83	8	93	477.104
Procyanidin B1	Bronze	0.81	11	1	577.136
Procyanidin B2	Bronze	0.71	9	14	575.108
Catechin-3-*O*-gallate	Bronze	0.81	8	2	441.083
Epicatechin-3-*O*-gallate	Bronze	0.71	8	10	487.088

MZErrorPPM: ppm error with the spectral library match, LibMZ: *m/z* value of the spectral library match.

Later on, all the major peaks detected were tentatively characterized by means of MS data, together with the interpretation of the observed MS/MS spectra in comparison with those found in the literature and the information derived from the FBMN analysis previously done and MS-DIAL software results. The formerly identified phytochemicals from the same botanical family or species were also utilized in the identification when applicable. This analysis allowed the identification of 13 phytochemical compounds from a total of 15 peaks. Four compounds were confirmed using authentic standards while the others were tentatively characterized. All of them were reported for the first time in *C. cowellii* leaves (Table 2).

Table 2. Chemical composition of the total extract from the leaves of *C. cowellii*.

Peak No.	Rt (min)	Measured mass (*m/z*)	Theoretical mass (*m/z*)	Accuracy (ppm)	MS/MS ions	MF	Tentative identification
1	2.03	169.0130	169.0137	-4.1	125.0268	$C_7H_5O_5$	Gallic acid (std)
2	6.04	289.0728	289.0712	5.5	125.8721	$C_{15}H_{13}O_6$	Catechin (std)
3	7.22	289.0693	289.0712	-6.6	245.0787/137.0222/125.0238	$C_{15}H_{13}O_6$	Epicatechin (std)
4	9.98	479.0845	479.0826	4.0	317.0249/316.0233/287.0161/271.0255	$C_{21}H_{19}O_{13}$	Myricetin-3-*O*-galactoside
5	10.21	729.1411	729.1456	-6.2	577.1219/451.1033/441.0815/407.0768/289.0728/287.0542	$C_{37}H_{29}O_{16}$	Procyanidin B1 monogallate
6	10.60	493.0612	493.0618	-1.2	317.0285/287.0196/178.9975	$C_{21}H_{17}O_{14}$	Myricetin-*O*-glucuronide
7	10.87	441.0815	441.0822	-1.6	289.0693/169.0157/125.0238	$C_{22}H_{17}O_{10}$	Epicatechin-3-*O*-gallate (std)
8	11.11	567.2066	567.2078	-2.1	341.1396/326.11326/160.8430	$C_{27}H_{35}O_{13}$	Unknown

9	11.29	463.0859	463.0877	-3.9	317.0285/316.0233 /271.0255	$C_{21}H_{19}O_{12}$	Myricetin-3-*O*-deoxyhexoside
10	11.43	463.0859	463.0877	-3.9	301.0344/300.0265 /271.0221	$C_{21}H_{19}O_{12}$	Quercetin-*O*-hexoside 1
11	11.58	463.0859	463.0877	-3.9	301.0344/300.0265 /271.0221	$C_{21}H_{19}O_{12}$	Quercetin-*O*-hexoside 2
12	12.02	477.0659	477.0669	-2.1	301.0344/299.0204 /271.0255	$C_{21}H_{17}O_{13}$	Quercetin-3-*O*-glucuronide
13	12.38	433.0745	433.0771	-6.0	301.0344/300.0265 /271.0255/255.0287	$C_{20}H_{17}O_{11}$	Quercetin-*O*-pentoside 1
14	12.51	433.0745	433.0771	-6.0	301.0344/300.0265 /271.0221/255.0287	$C_{20}H_{17}O_{11}$	Quercetin-*O*-pentoside 2
15	17.12	331.2498	331.2484	4.2	313.2348/160.8430	$C_{18}H_{35}O_5$	Unknown

Rt, retention time; MF, molecular formula. (std) The compound was also identified by comparing the chromatographic behavior with the authentic standard.

Conclusions

Using UHPLC-ESI-QTOF-MS and supported by FBMN analysis, thirteen metabolites were detected from the leaves of the endemic Cuban plant *Coccoloba cowellii*, including gallic acid, catechin, epicatechin and epicatechin-3-*O*-gallate. This report could contribute for the better understanding of the phytochemistry in the genus *Coccoloba*, increasing the interest in *C. cowellii* species and encouraging the implementation of future conservation strategies.

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