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molecules
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Chemical fingerprinting and antimicrobial evaluation of the methanolic extract of the leaves of the endemic Cuban plant Coccoloba cowellii

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Introduction

The genus *Coccoloba* comprises approximately 120-150 species of flowering plants from the subfamily Erigonoideae of the Polygonaceae, order Caryophyllales. In Cuba, the presence of 34 species of *Coccoloba* has been informed [1]. From them, 25 are recognized as endemic. Nevertheless, just informs of *C. uvifera* ethnopharmacological uses was found in the consulted bibliography related to the treatment of sores and grains, hoarseness, asthma, dysentery, anti-hemorrhagic and body itching [2].

One of the almost unknown endemic plant of this genus that grow up in Cuba is *Coccoloba cowellii* Britton, which classifies as critically endangered (CR) according to the International Union for Conservation of Nature (IUCN) [3]. Only preliminary information is known about the chemical composition of this plant and it antioxidant activity [4]. This gap in the knowledge of this species which risk of disappearing without having been explored in their potential, led us to the development of this research. With this intention, 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. Microanalytical pharmacological tests were also considered with this conservation purpose.

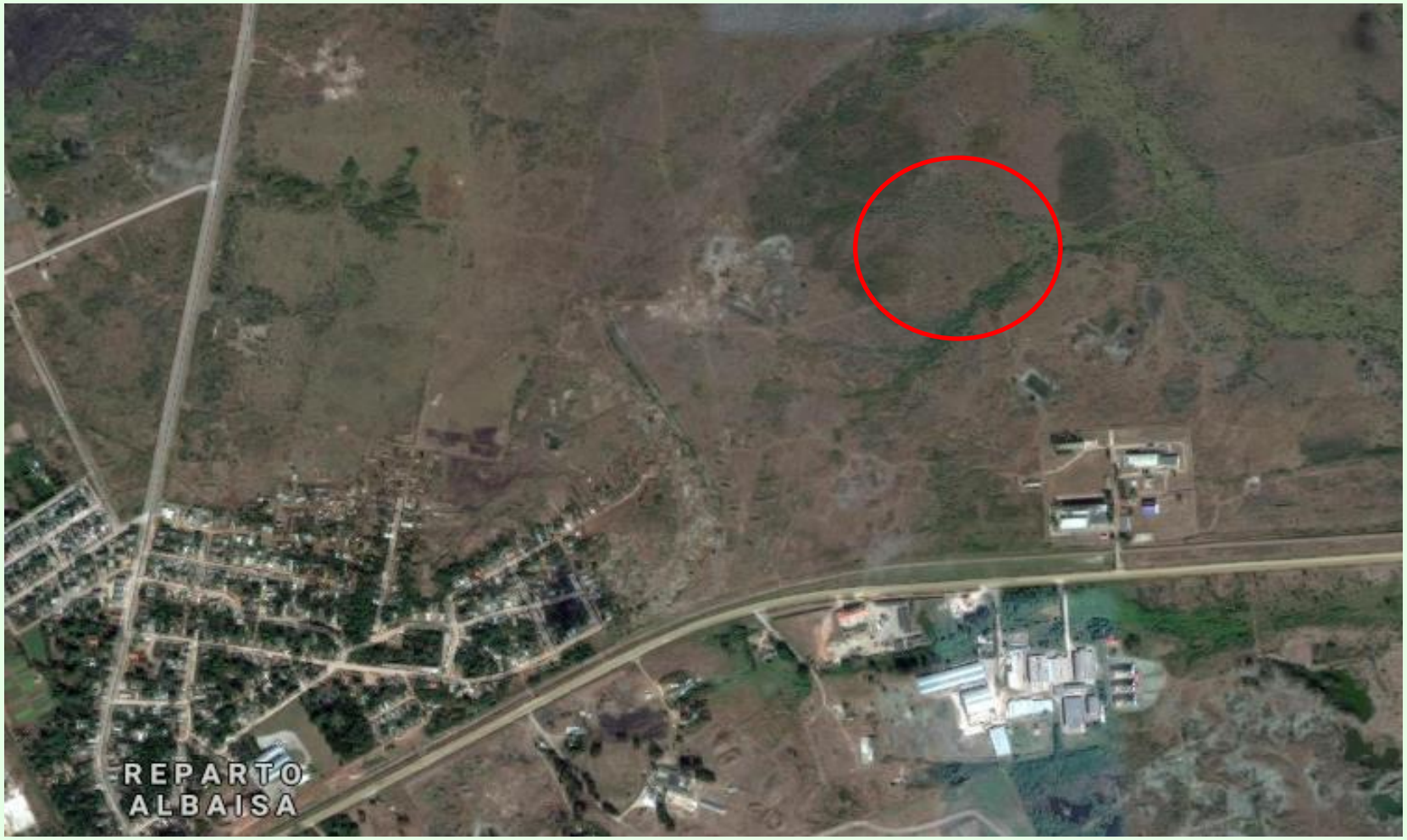
1. Noa, I.C. *Coccoloba howardii (Polygonaceae)*, a new species from Cuba. Willdenowia 2012, 42, 95–98, doi:10.3372/wi.42.42112.
2. Roig y Mesa, J.T. *Plantas medicinales, aromáticas o venenosas de Cuba (Tomo II)*; 2nd ed.; Editorial Científico-Técnica: La Habana, 2012; ISBN 978-959-05-0814-1.
3. González-Torres, L; Palmarola, A; González-Oliva, L; Bécquer, E; Testé, E. and Barrios, D. (Eds. . *Lista Roja*; 2016; Vol. 10; ISBN 978-959-300-113-7.
4. Méndez Rodríguez, D.; Molina Pérez, E.; Spengler Salabarria, I.; Escalona-Arranz, J.C.; Cos, P. Chemical composition and antioxidant activity of Coccoloba cowellii Britton Lic. *Rev. Cuba. Química* 2019, 32, 185–198.

Introduction

Coccoloba cowellii Britton

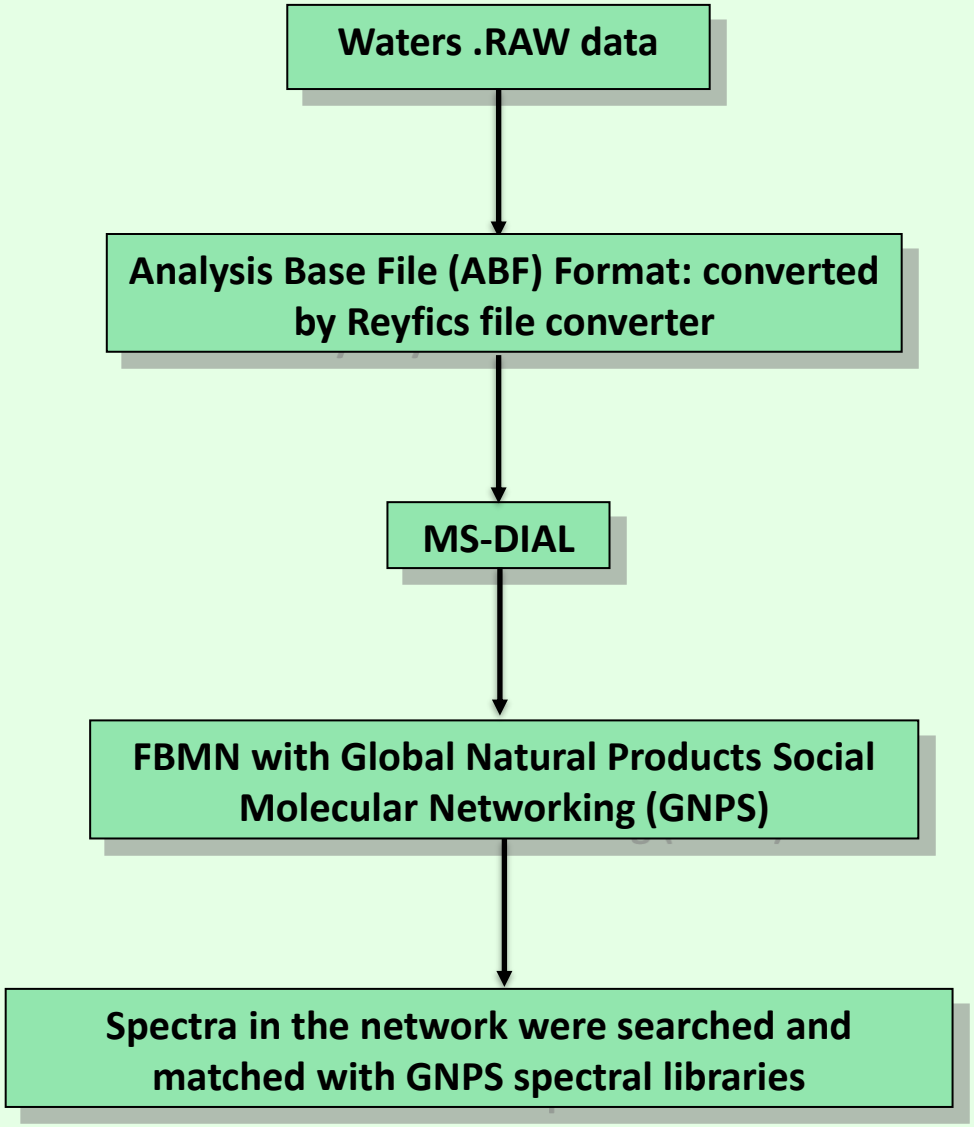
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Caryophyllales
Family: Polygonaceae
Subfamily: Eriogonoideae
Genus: *Coccoloba*
Species: *Coccoloba cowellii* Britton

Collection of plant material



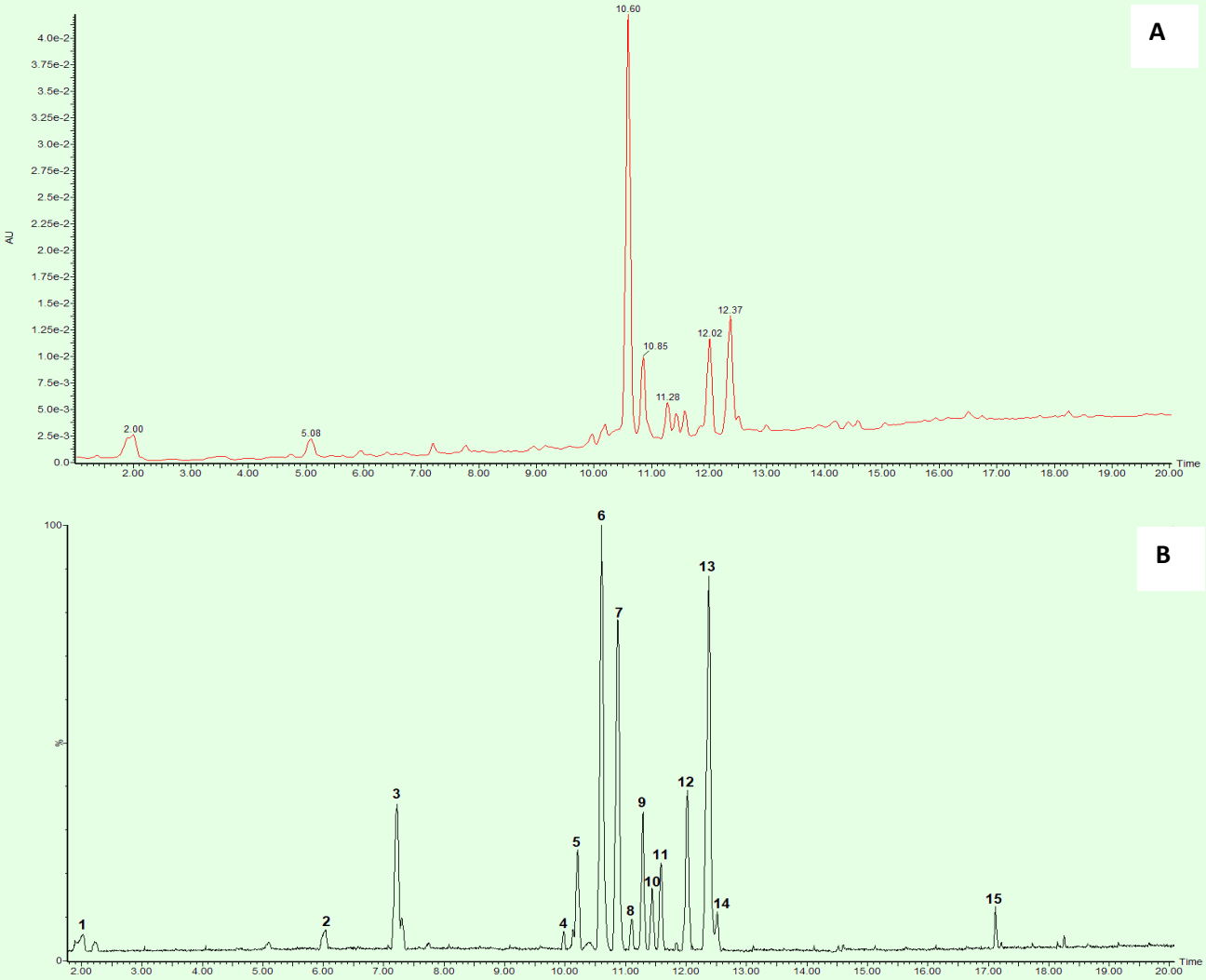
The plant material (*Coccoloba cowellii* leaves) was collected in July 2019, near the town of Albaisa, Camagüey province (Lat 21.43615, Lon -77.83253).

Dereplication strategy for the leaves extract of *C. cowellii*



Results

HPLC-DAD/QTOF-MS of the total extract of *C. cowellii*



Base peak intensity (BPI) chromatograms of: (**A**) UV at 280 nm and (**B**) MS in negative ion mode for the methanolic extract of *C. cowellii* leaves.

Results

Dereplication strategy for the methanolic extract of *C. cowellii*

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

Compound name	Library class	cosine	MZErrorPPM	LibMZ
Quercetin-3-O-rhamnoside (Quercitrin)	Bronze	0.85	1	447.093
Quercetin-3-O-galactoside (Hyperoside)	Bronze	0.80	0	463.088
Quercetin-3-O-arabinoside (Avicularin)	Bronze	0.72	0	433.078
Quercetin-3-O-glucuronide	Bronze	0.84	1	477.067
Quercetin 3-(2-galloylglucoside)	Bronze	0.73	37	615.099
Myricetin-3-O-pentoside	Bronze	0.85	10	449.067
Myricetin-3-O-galactoside	Bronze	0.93	2	479.083
4'-O-Methylmyricetin-3-O-rhamnoside (Mearnsitrin)	Gold	0.83	93	477.104
Procyanidin B1	Bronze	0.81	1	577.136
Procyanidin B2	Bronze	0.71	14	575.108
Catechin-3-O-gallate	Bronze	0.81	2	441.083
Epicatechin-3-O-gallate	Bronze	0.71	10	487.088

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

Results

HPLC-DAD/QTOF-MS of the methanolic extract of *C. cowellii*

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

Peak No.	Rt (min)	[M-H]- (m/z)	MS/MS ions	Acc Mass	ppm	MF	Tentative identification
1	2.03	169	125	169.0130	-4.1	$C_7H_5O_5$	Gallic acid (std)
2	6.04	289	245/165/137	289.0693	-6.6	$C_{15}H_{13}O_6$	Catechin (std)
3	7.22	289	245/205/179/165/137/125	289.0693	-6.6	$C_{15}H_{13}O_6$	Epicatechin (std)
4	9.98	479	317/316/287/271	479.0845	4.0	$C_{21}H_{19}O_{13}$	Myricetin-3-O-hexoside
5	10.21	729	577/451/441/407/289/287	729.1466	1.4	$C_{37}H_{29}O_{16}$	B-type procyanidin monogallate
6	10.60	493	317/287/179	493.0612	-1.2	$C_{21}H_{17}O_{14}$	Myricetin glucuronide
7	10.87	441	289/169/125	441.0815	-0.7	$C_{22}H_{17}O_{10}$	Epicatechin-3-O-gallate (std)
8	11.11	567	341/326/161	567.2066	-2.1	$C_{27}H_{35}O_{13}$	Unknown
9	11.29	463	317/316/287/271	463.0859	-3.9	$C_{21}H_{19}O_{12}$	Myricetin deoxyhexoside
10	11.43	463	301/300/271	463.0859	-3.9	$C_{21}H_{19}O_{12}$	Quercetin hexoside 1
11	11.58	463	301/300/271	463.0859	-3.9	$C_{21}H_{19}O_{12}$	Quercetin hexoside 2
12	12.02	477	301/271	477.0659	-2.1	$C_{21}H_{17}O_{13}$	Quercetin glucuronide
13	12.38	433	301/300/271/255	433.0745	-6.0	$C_{20}H_{17}O_{11}$	Quercetin pentoside 1
14	12.51	433	301/300/271	433.0745	-6.0	$C_{20}H_{17}O_{11}$	Quercetin pentoside 2
15	17.12	331	313/161	331.2498	4.2	$C_{18}H_{35}O_5$	Unknown

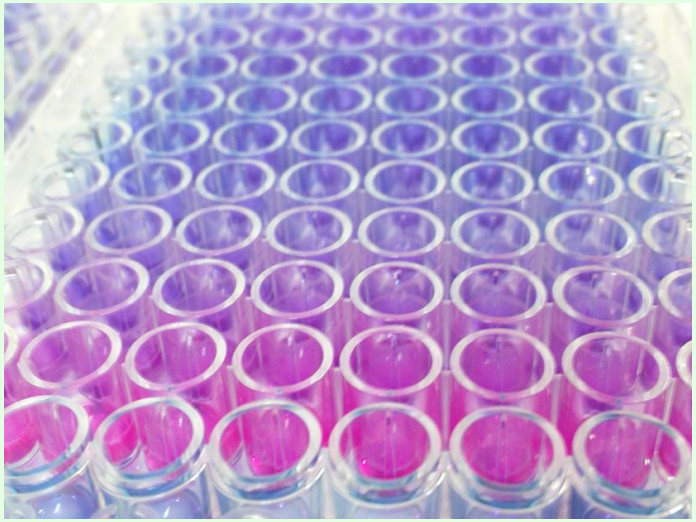
Rt, retention time; Acc Mass, accurate mass; ppm, error associated with the accurate mass; MF, molecular formula. $^{(std)}$ The compound was also identified by comparing the chromatography behaviour with the authentic standards.

Results

Antimicrobial screening of the methanolic extract of *C. cowellii*

Test sample	Cytotoxicity (IC50 µg/mL)	Antimicrobial screening (IC50 µg/mL)						
	MRC-5 SV2	*S. aureus*	*E. coli*	*C. albicans*	*A. fumigatus*	*C. neoformans*	*T. cruzi*	*T. brucei*
ME	>64.00	>64.00	>64.00	1.68±0.64	>64.00	2.69±2.02	38.38±6.82	33.12±0.38

ME: methanolic extract. MRC-5 SV2: Human fetal lung fibroblasts; *S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli*; *C. albicans*: *Candida albicans*; *A. fumigatus*: *Aspergillus fumigatus*; *C. neoformans*: *Cryptococcus neoformans*; *T. cruzi*: *Trypanosoma cruzi*; *T. brucei*: *Trypanosoma brucei*. Reference compounds: Tamoxifen (MRC-5 SV2) IC50 10.49 µM; Doxycycline (*S. aureus*) IC50 0.18 µM; Doxycycline (*E. coli*) IC50 0.60 µM; Flucytosine (*C. albicans*) IC50 0.61 µM; Econazole (*A. fumigatus*) IC50 0.74 µM; Miconazole (*C. neoformans*) IC50 0.15 µM; Benznidazole (*T. cruzi*) IC50 3.13 µM; Suramine (*T. brucei*) IC50 0.05 µM.



Conclusions

Thirteen metabolites were detected from the methanolic extract of the leaves of the endemic Cuban plant *Coccoloba cowellii*, using UHPLC-ESI-QTOF-MS analysis, including gallic acid, catechin, epicatechin and epicatechin-3-O-gallate. *C. cowellii* showed a good antifungal activity against *Candida albicans* and *Cryptococcus neoformans* and a moderate activity against parasites. This report could contribute for the better understanding of chemistry and biological activities in the genus *Coccoloba*, increasing the interest in *C. cowelli* plants and encouraging the implementation of future conservation strategies. Other studies are being carried out to corroborate these results and determine the metabolites responsible for the aforementioned activities.

Acknowledgments

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