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Chemical constituents and antifungal activity of *Coccoloba cowellii* leaves

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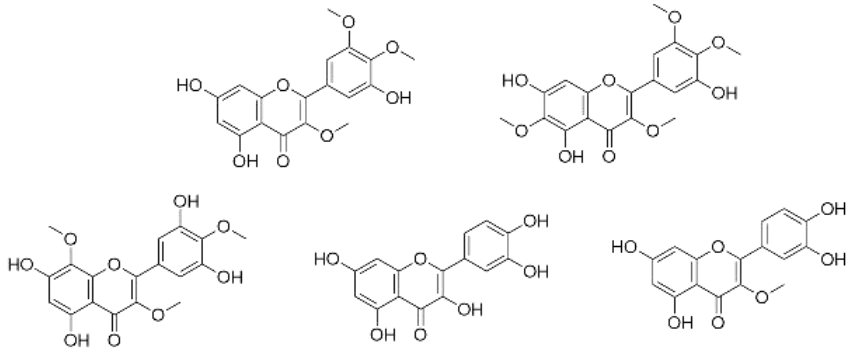
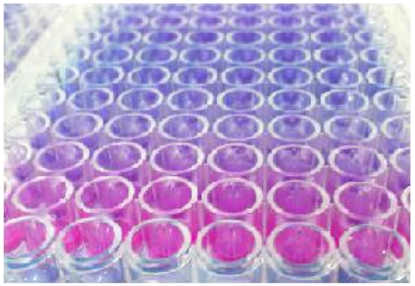
Coccoloba cowellii Britton





Good antifungal activity against *Candida* spp.

Abstract: The genus *Coccoloba* (Polygonaceae, order Caryophylalles) comprises approximately 150 species of flowering plants. *Coccoloba cowellii* Britton is an endemic and critically endangered representative of this genus that only grows in the municipality of Camagüey, province of Cuba. Preliminary investigation of its total methanolic extract led to the discovery of promising antifungal activity. In the present study, a bioassay-guided fractionation by means of flash chromatography and employing 1D and 2D NMR, and NMR-based machine learning tool "Small Molecule Accurate Recognition Technology" (SMART 2.1, available at https://smart.ucsd.edu/classic), allowed the isolation of quercetin and four methoxyflavonoids: 3-O-methylquercetin, myricetin 3,3',4'-trimethyl ether, 6-methoxymyricetin 3,4'-dimethyl ether, and 6-methoxymyricetin 3,3',4'-trimethyl ether. The leaf extract, fractions, and compounds were tested against various fungi, and showed a strong antifungal effect against *Cryptococcus neoformans* and various *Candida* spp. with no cytotoxicity (CC50 > 64.0 µg/mL) on human foetal lung fibroblasts (MRC-5 SV2 cells), determined by the resazurin assay. The total methanolic extract showed higher and broader activity when compared with the fractions and mixture of compounds.

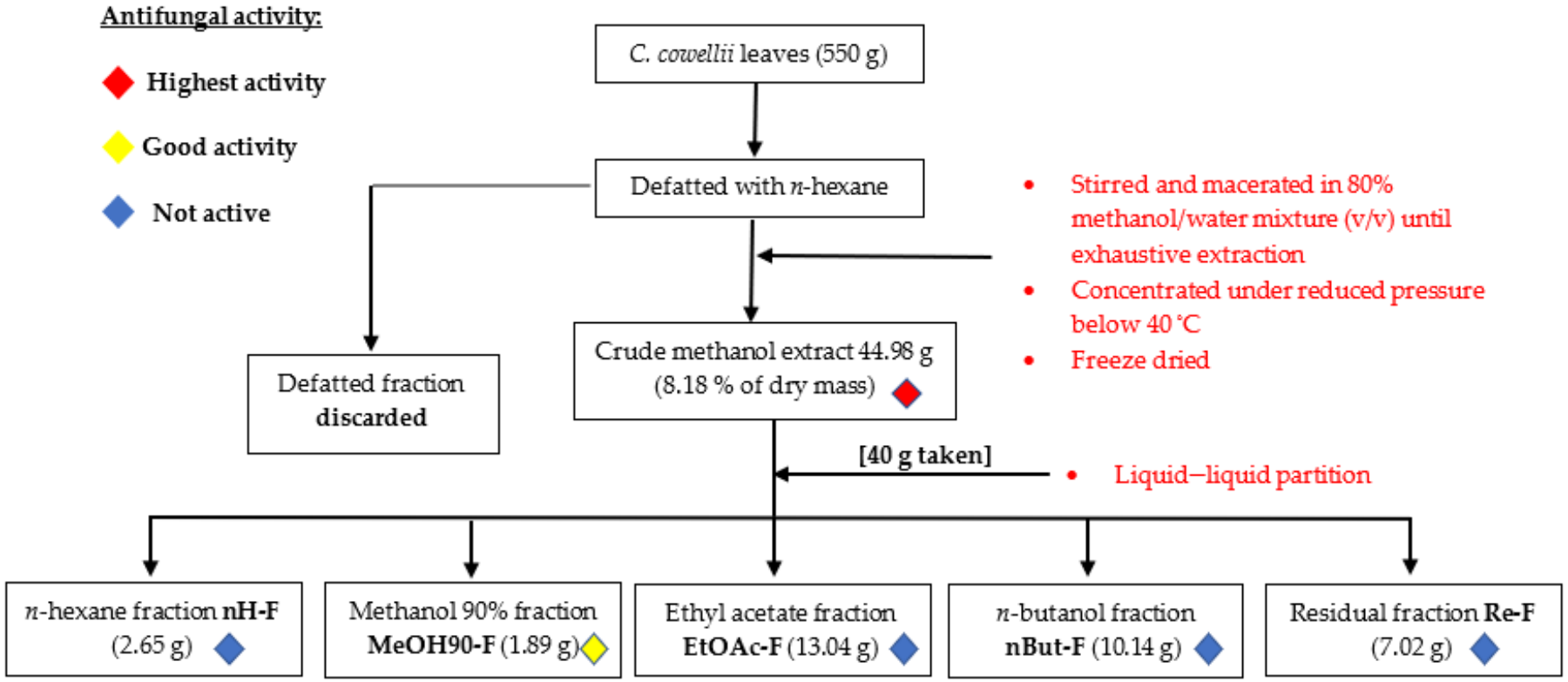
Keywords: antifungal; *Coccoloba cowellii*; methoxyflavonoids; Polygonaceae; UHPLC-ESI-QTOF-MS.

Introduction

Growth environment (municipality of Camagüey, Cuba) and flowering branch of *C. cowellii* Britton

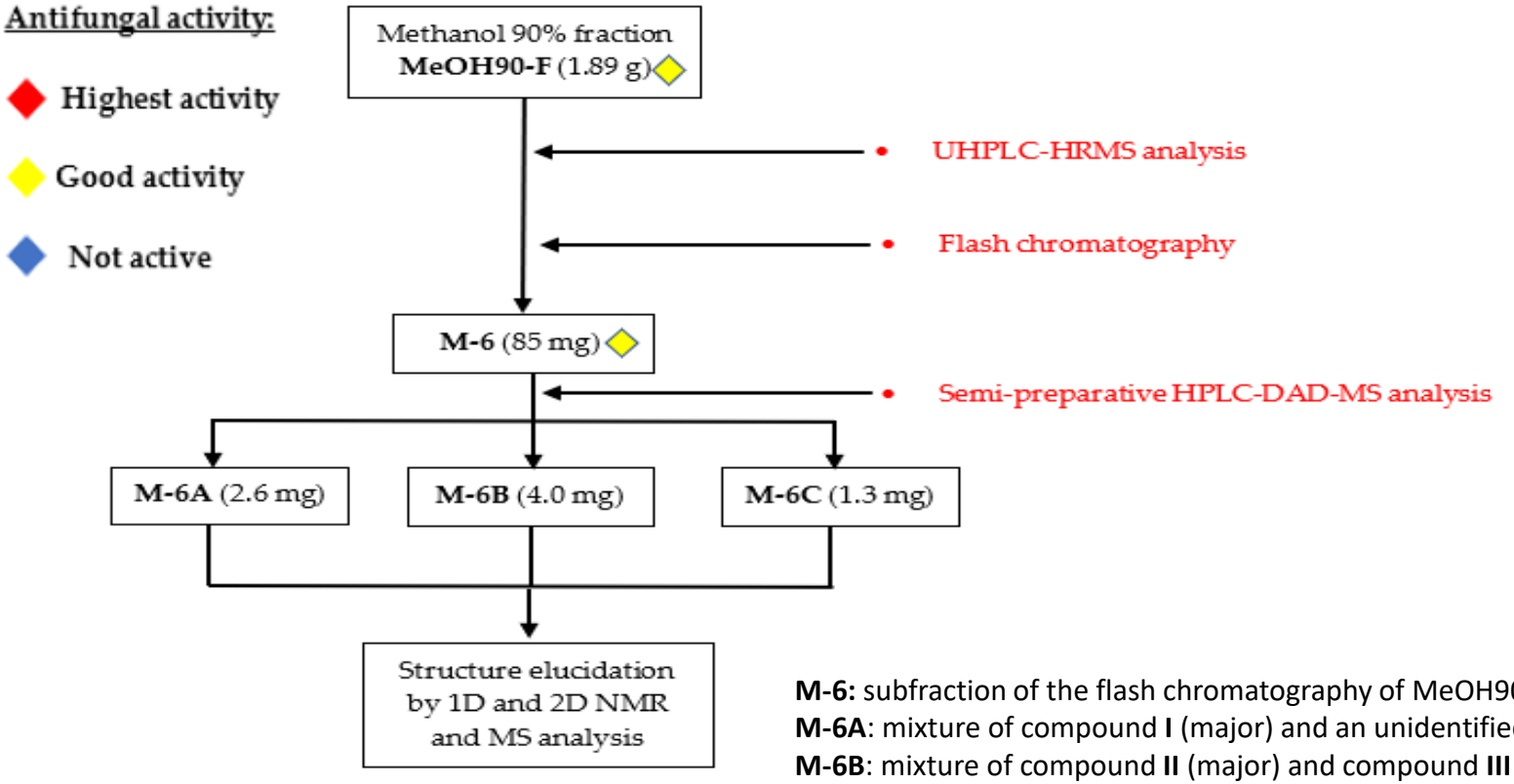
Introduction

General scheme of the bioassay-guided fractionation performed on *C. cowellii* leaves



Introduction

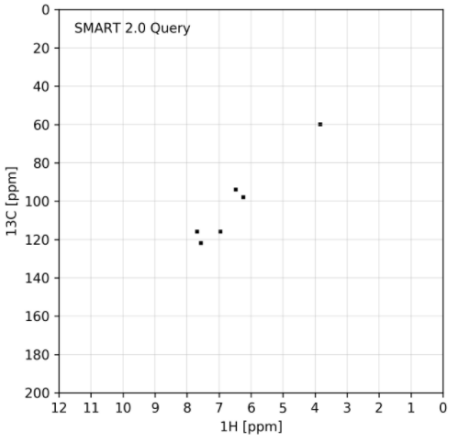
General scheme of the bioassay-guided fractionation performed on *C. cowellii* leaves



M-6: subfraction of the flash chromatography of MeOH90-F.
M-6A: mixture of compound **I** (major) and an unidentified impurity (minor).
M-6B: mixture of compound **II** (major) and compound **III** (minor).
M-6C: mixture of compound **IV** (major) and compound **V** (minor).

Introduction

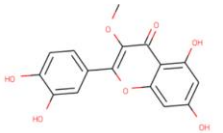
Prediction of chemical structures from NMR data



^{1}H-^{13}C-HSQC spectra.

SMART

Small Molecule Accurate Recognition Technology.

Name	Structure	Cosine score	MW	Source	External
3-O-Methylquercetin		0.9794441055780836	316.1	ACD_Labs	GNPS

These proposed results were contrasted with those obtained from mass spectra derived from the semi-preparative HPLC-DAD-MS system.

Possible structure.

Results and discussion

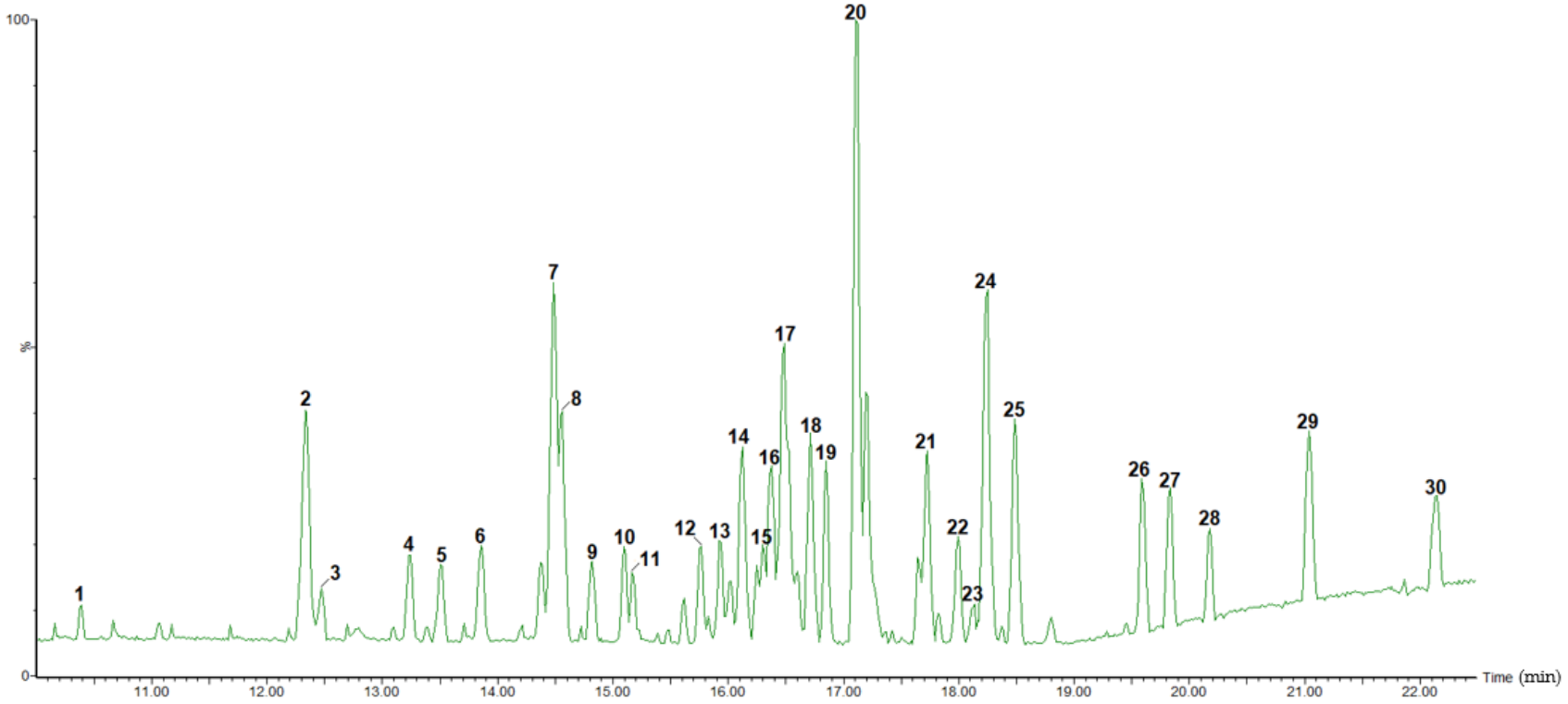
Table 1. *In vitro* antifungal and cytotoxic activity of the total extract and fractions from *C. cowellii* leaves.

Test Sample	Cytotoxicity (CC_{50} µg/mL)	Antifungal Screening (IC_{50} µg/mL)					
	MRC-5	*Aspergillus fumigatus*	*Cryptococcus neoformans*	*Candida albicans*	*Candida parapsilosis*	*Candida glabrata*	*Candida tropicalis*
TE	>64.0	>64.0	2.7 ± 2.0	1.7 ± 0.6	8.5 ± 0.5	0.4 ± 0.0	21.2 ± 1.8
MeOH90-F	>64.0	>64.0	10.5 ± 1.0	8.3 ± 0.9	13.3 ± 1.1	2.4 ± 0.4	>64.0
nH-F	29.3 ± 1.5	Nd	>64.0	>64.0	Nd	Nd	Nd
EtOAc-F	>64.0	Nd	>64.0	>64.0	Nd	Nd	Nd
nBut-F	>64.0	Nd	>64.0	>64.0	Nd	Nd	Nd
Re-F	>64.0	Nd	>64.0	>64.0	Nd	Nd	Nd
Miconazole	19.8 ± 0.7	3.7 ± 0.5	0.2 ± 0.0	3.4 ± 0.2	1.1 ± 0.1	0.2 ± 0.0	3.6 ± 1.0

TE: total extract; MeOH90-F: methanol 90% fraction; nH-F: n-hexane fraction; EtOAc-F: ethyl acetate fraction; nBut-F: n-butanol fraction; Re-F: residual fraction; MRC-5: human foetal lung fibroblasts; Nd: not determined. Values are means ± SD of three replicates.

Results and discussion

HPLC-DAD/QTOF-MS of the methanol 90% fraction from *C. cowellii*



Base peak intensity (BPI) chromatogram (in negative ion mode) of the MeOH90-F fraction.

Results and discussion

Table 2. Chemical composition of the methanol 90% fraction of the total extract of *C. cowellii*. (part 1)

Peak No.	Rt (min)	$[M-H]^-$ (m/z)	Theoretical Mass (m/z)	Accuracy (ppm)	MS/MS Ions	MF	Tentative Identification
1	10.39	493.0620	493.0618	0.4	317.0281/315.0105/ 287.0563/178.9872	$C_{21}H_{17}O_{14}$	Myricetin-O-glucuronide
2	12.33	433.0775	433.0771	0.9	301.0344/300.0273/ 271.0247/255.0294	$C_{20}H_{17}O_{11}$	Quercetin-O-pentoside 1
3	12.47	433.0763	433.0771	−1.8	301.0357/300.0253/ 271.0255/255.0187	$C_{20}H_{17}O_{11}$	Quercetin-O-pentoside 2
4	13.23	555.2225	555.2230	−0.9	507.2011/477.1888	$C_{30}H_{35}O_{10}$	Trilignol G(8–O–4)G(8–5)G
5	13.50	555.2216	555.2230	−2.5	507.1984/477.1816/ 341.1288/329.1320/ 195.0650/165.0273	$C_{30}H_{35}O_{10}$	Trilignol G(8–O–4)G(8–5)G
6	13.85	312.1228	312.1236	−2.6	197.8091/195.8118/ 116.9287	-	Unknown
7	14.49	585.2429	585.2430	−0.2	537.2122/507.1984/ 371.1458/359.1454/ 195.0658/165.0374	$C_{31}H_{37}O_{11}$	Trilignol G(8–O–4)X(8–8)X
8	14.56	583.2163	583.2179	−2.7	535.1965/505.1852/ 369.1333/357.1330/ 195.0658/165.0301	$C_{31}H_{35}O_{11}$	Trilignol G(8–O–4)S(8–5)G
9	14.82	585.2329	585.2336	−1.2	537.2112/507.1821/ 359.1410/195.0639/ 165.0157	$C_{31}H_{37}O_{11}$	Trilignol G(8–O–4)X(8–8)X

Rt, retention time; MF, molecular formula.

Results and discussion

Table 2. Chemical composition of the methanol 90% fraction of the total extract of *C. cowellii*. (part 2)

Peak No.	Rt (min)	$[M-H]^-$ (m/z)	Theoretical Mass (m/z)	Accuracy (ppm)	MS/MS Ions	MF	Tentative Identification
10	15.09	585.2331	585.2336	−0.9	537.2020/507.1826/ 371.1437/359.1445/ 195.0655/165.0552	$C_{31}H_{37}O_{11}$	Trilignol G(8–O–4)X(8–8)X
11	15.17	583.2172	583.2179	−1.2	369.1325/357.1325/ 195.0656/165.0551	$C_{31}H_{35}O_{11}$	Trilignol G(8–O–4)S(8–5)G
12	15.76	583.2177	583.2179	−0.3	565.2036/489.1883/ 477.1877/417.1481/ 371.1414/359.1383/ 193.0497	-	Unknown
13	16.02	583.2177	583.2179	−0.3	581.1965/535.1947/ 387.1389/367.1148/ 195.0648/165.0052	-	Unknown
14	16.12	315.0513	315.0505	2.5	300.0270/271.0238	$C_{16}H_{11}O_7$	3-*O*-Methylquercetin
15	16.24	375.0704	375.0716	−3.2	360.0495/345.0239/ 330.0117/327.1691/ 317.0265/300.0250/ 171.0929	$C_{18}H_{15}O_9$	6-Methoxymyricetin 3,4'-dimethyl ether
16	16.37	327.2177	327.2171	1.8	285.0412/256.0378/ 229.1443/211.1334/ 171.1033	-	Unknown

Rt, retention time; MF, molecular formula.

Results and discussion

Table 2. Chemical composition of the methanol 90% fraction of the total extract of *C. cowellii*. (part 3)

Peak No.	Rt (min)	$[M-H]^-$ (m/z)	Theoretical Mass (m/z)	Accuracy (ppm)	MS/MS Ions	MF	Tentative Identification
17	16.49	345.0612	345.0610	0.6	301.0422	-	Unknown
18	16.71	315.0510	315.0505	1.6	300.0278/271.0252/255.0304/243.0296	$C_{16}H_{11}O_7$	*O*-Methylquercetin
19	16.85	809.3019	809.3021	−0.2	761.2747/613.2260/565.2047/417.1499/195.0660	$C_{42}H_{49}O_{16}$	Tetralignol G(8–O–4)G(8–O–4)S(8–8)S
20	17.11	331.2645	331.2637	2.4	313.2187	-	Unknown
21	17.73	389.0888	389.0873	3.9	374.0632/359.0416/331.0509/316.0201/287.2135	$C_{19}H_{17}O_9$	6-Methoxymyricetin 3,3',4'-trimethyl ether
22	17.99	359.0764	359.0767	−0.8	344.0509/329.0413/301.0361/286.0089/273.0367/257.9776/242.0100/222.9688/162.8474	$C_{18}H_{15}O_8$	Myricetin 3,3',4'-trimethyl ether
23	18.13	359.0751	359.0767	−4.5	344.0493/329.0565/301.0364/286.0081/257.9645/222.9675/162.8543	$C_{18}H_{15}O_8$	Methoxyquercetin dimethyl ether 1

Rt, retention time; MF, molecular formula.

Results and discussion

Table 2. Chemical composition of the methanol 90% fraction of the total extract of *C. cowellii*. (part 4)

Peak No.	Rt (min)	$[M-H]^-$ (m/z)	Theoretical Mass (m/z)	Accuracy (ppm)	MS/MS Ions	MF	Tentative Identification
24	18.25	389.0870	389.0873	−0.8	374.0634/359.0398/344.0168/316.0218/300.9995/245.0086	$C_{19}H_{17}O_9$	Methoxymyricetin trimethyl ether
25	18.48	359.0771	359.0767	1.1	344.0535/329.0302/301.0346/286.0122/258.0163	$C_{18}H_{15}O_8$	Methoxyquercetin dimethyl ether 2
26	19.58	403.1047	403.1029	4.5	388.0773/373.0557/358.0301/345.0566/330.0363/315.0175/257.9344/222.9669	$C_{20}H_{19}O_9$	Methoxymyricetin tetramethyl ether
27	19.83	373.0939	373.0923	4.3	358.0623/343.0453/328.0199/315.0660/300.0232/285.0035/257.9385/222.9662	$C_{19}H_{17}O_8$	Myricetin tetramethyl ether
28	20.18	349.2156	349.2168	−3.4	313.2335/251.1598/199.8060/197.8089/195.8118/116.9286	-	Unknown
29	21.03	721.3658	721.3647	1.5	675.3555/415.1435/397.1342/277.1996/257.9326/222.9646	-	Unknown
30	22.15	559.3133	559.3118	2.7	567.2234/505.1054/320.0494/277.2092/257.9327/222.9659	-	Unknown

Rt, retention time; MF, molecular formula.

Results and discussion

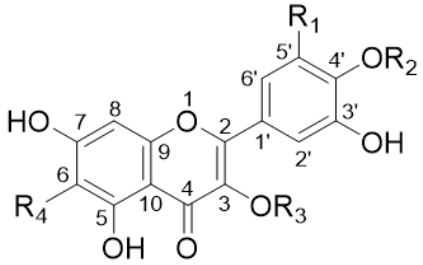
Table 3. *In vitro* antifungal and cytotoxic activity of subfraction M-6 and the binary mixtures M-6A, M-6B, and M-6C from *C. cowellii* leaves.

Test Sample	Cytotoxicity (CC_{50} µg/mL)	Antifungal Screening (IC_{50} µg/mL)					
	MRC-5	*A. fumigatus*	*C. neoformans*	*C. albicans*	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*
M-6	>64.0	>64.0	50.3 ± 9.2	>64.0	>64.0	9.5 ± 1.1	>64.0
M-6A	>64.0	>64.0	>64.0	>64.0	>64.0	7.9 ± 1.3	>64.0
M-6B	>64.0	>64.0	59.5 ± 6.4	>64.0	>64.0	9.1 ± 1.8	>64.0
M-6C	>32.0	>32.0	8.3 ± 0.0	>32.0	>32.0	3.8 ± 0.0	>32.0
Miconazole	19.8 ± 0.7	3.7 ± 0.5	0.2 ± 0.0	3.4 ± 0.2	1.1 ± 0.1	0.2 ± 0.0	3.6 ± 1.0

M-6: subfraction of the flash chromatography of MeOH90-F; M-6A: compound **I** and minor impurity; M-6B: mixture of compounds **II** and **III**; M-6C: mixture of compounds **IV** and **V**. MRC-5: human foetal lung fibroblasts. Values are means ± SD of three replicates..

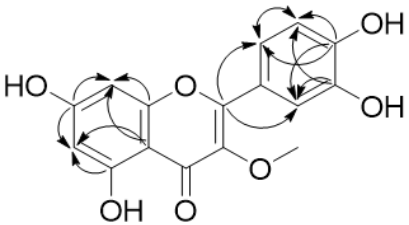
Results and discussion

Compounds isolated from the methanol 90% fraction of *C. cowellii*



Compound	R_1	R_2	R_3	R_4
I	-H	-H	-H	-H
II	-H	-H	$-CH_3$	-H
III	-OH	$-CH_3$	$-CH_3$	$-OCH_3$
IV	$-OCH_3$	$-CH_3$	$-CH_3$	$-OCH_3$
V	$-OCH_3$	$-CH_3$	$-CH_3$	-H

(a)



(b)

(a) Structures of compounds isolated from leaves of *C. cowellii*.
(b) Correlations $^2J_{H\text{-}C}$ and $^3J_{H\text{-}C}$ observed in the HMBC spectra of compound **II**.

Conclusions

In this study, five secondary metabolites were isolated and characterised from the MeOH90-F fraction of the total extract of *C. cowellii* by means of a combined methodology of NMR and MS analysis. All five are reported here for the first time for both the plant and the genus. Another 16 compounds were tentatively characterised employing UHPLC-HRMS. *C. cowellii* extract was confirmed to have good antifungal activity against a second fungal/yeast panel, while fractions and mixtures of compounds obtained from the bioassay-guided fractionation showed acceptable activity specifically against *C. glabrata* and C. *neoformans.* These results highlight the possible use of this plant as a natural antifungal and contribute to a better understanding of the phytochemistry and biological activities of the genus *Coccoloba.*

Acknowledgments

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