

Bioassay-guided metabolomic profiling reveals flavanone-rich fractions as antibacterial agents in *Metrodorea nigra* (Rutaceae)

Authors

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Affiliations

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Supplementary information (SI)

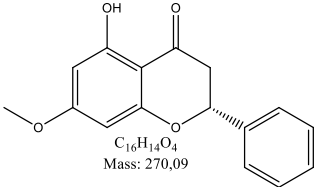
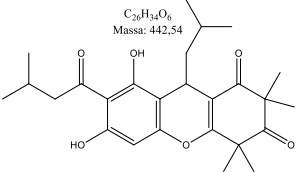
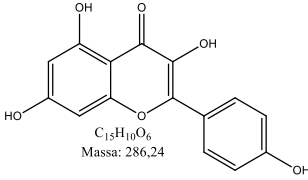
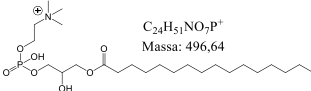
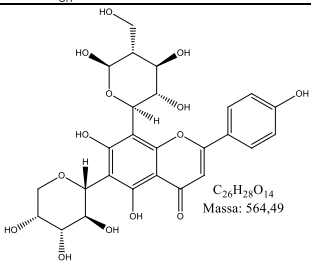
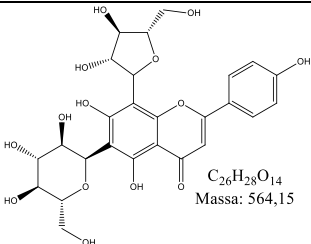
Supplementary Table 1: Selected ATCC strains used in antimicrobial assays

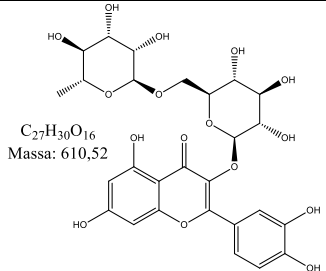
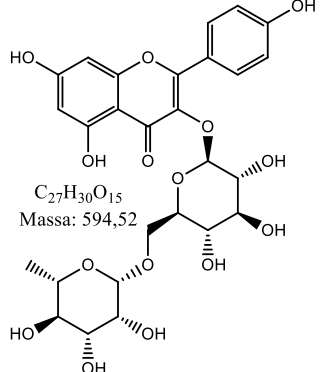
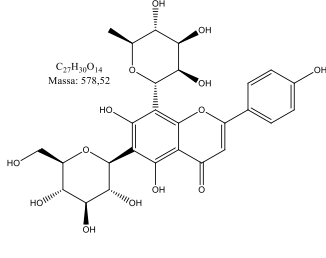
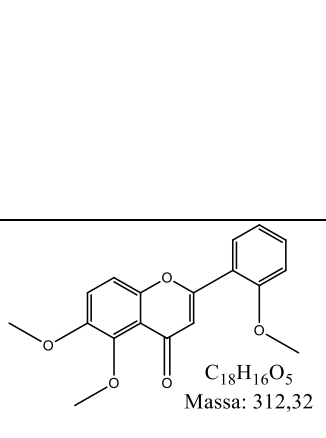
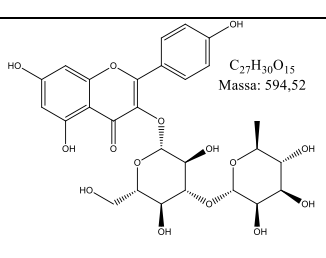
Bacterium	Strain	Gram	Ciprofloxacin resistance	Oxacillin resistance	Methicillin resistance	Another antimicrobials
<i>Staphylococcus aureus</i>	ATCC 33591	Positive (+)	-/-	4 µg/ml	Resistant	-/-
	ATCC 29213		-/-	0.5 µg/ml	Sensible	Sensible to vancomycin and ceftalorin
	ATCC 25923		-/-	Sensible	-/-	-/-
<i>Pseudomonas aeruginosa</i>	ATCC 27853	Negative (-)	0.125 µg/ml	-/-	-/-	-/-
<i>Staphylococcus saprophyticus</i>	ATCC 15305	Positive (+)	-/-	2 µg/ml	Resistant	-/-
<i>Staphylococcus epidermidis</i>	ATCC 12228	Positive (+)	-/-	Sensible ≤ 0.25 µg/ml	Sensible	-/-
	ATCC 35984		0,0625 µg/ml	2 µg/ml	-/-	-/-
<i>Klebsiella pneumoniae</i>	ATCC 700603	Negative (-)	-/-	-/-	-/-	Resistant to Ceftazidim and beta-lactamics

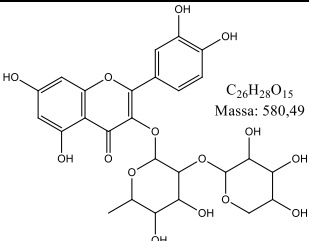
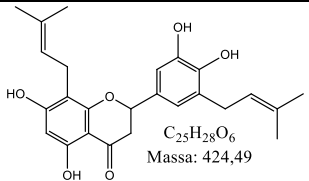
Supplementary Table 2: Selected clinical strains used in antimicrobial assays

Bacterium	Clinic strain	Gram	Obtained source	Ciprofloxacin resistance	Oxacillin resistance	Penicillin resistance
<i>Staphylococcus aureus</i>	517	Positive (+)	Blood	-/-	Resistant	Resistant
	664		Skin wound	-/-	Resistant	Resistant
	1025		Prosthesis	-/-	Sensible	-/-
<i>Pseudomonas aeruginosa</i>	i059	Negative (-)	Catheter tip	Sensible	-/-	-/-

Supplementary Table 3: Molecules annotated in *M. nigra* fractions using GNPS library search tool.

Assignment	Rt (min)	Molecular Ion [M ± H] - m/z	MS/MS Fragment Ions - m/z	Chemical structure	Origin fraction	Ionization Mode	Class
5-hydroxy-7-methoxy flavanone pinostrobin (5)	14.61	271.2	167 (100%), 131, 103		Sp1D subfraction	Positive	Flavonoid
6,8-dihydroxy-2,2,4,4-tetramethyl-7-(3-methylbutanoyl)-9-(2-methylpropyl)-9H-xanthene-1,3-dione rhodomyrthone (6)	10.49	441.2	383, 369 (100%), 351, 335, 351, 233		Sp1D subfraction	Negative	Xanthone
3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one kaempferol (7)	9.36	278.0	269, 258, 241, 231, 213 (100%), 165, 153, 121		Ethanollic Crude Extract // Ethyl acetate Fraction	Positive and negative	Flavonoid
palmitoylglycerophosphocholine (8)	8.27	496.3	478 (100%), 184		Dichloromethane fraction	Positive	Fatty acid
isoshaftoside (9)	8.6	565.0	547 (100%), 529, 511, 499, 481, 445		Ethanollic Crude Extract // Ethyl acetate Fraction	Positive and negative	Glycosylated Flavonoid
Massbank:PR303329 NP-000004(11) (10)	8.6	565.0	547 (100%), 529, 511, 499, 481, 457, 445, 427, 409		Ethanollic crude extract	Positive	Glycosylated Flavonoid

<p>rutin (11)</p>	9.03	609.1	373, 367, 353, 343, 301 (100%), 271, 255	 <p>$C_{27}H_{30}O_{16}$ Massa: 610,52</p>	Ethyl acetate Fraction	Negative	Glycosyla ted Flavonoid
<p>kaempferol-3- O-rutinoside (12)</p>	9.34	593.1	327, 285 (100%), 255	 <p>$C_{27}H_{30}O_{15}$ Massa: 594,52</p>	Ethyl acetate Fraction	Negative	Glycosyla ted Flavonoid
<p>5,7-dihydroxy- 2-(4- hydroxyphenyl) -6- [(2S,3R,4R,5S,6 R)-3,4,5- trihydroxy-6- (hydroxymethyl)oxan-2-yl]-8- [(2S,3R,4R,5R, 6S)-3,4,5- trihydroxy-6- methyloxan-2- yl]chromen-4- one (13)</p>	9.08	577.2	529, 503, 487, 473, 457, 413, 395, 383, 353 (100%), 325, 311, 297	 <p>$C_{27}H_{30}O_{14}$ Massa: 578,52</p>	Ethanolic Crude Extract // Ethyl acetate Fraction	Negative	Glycosyla ted Flavonoid
<p>5,6,2- Trimethoxyflav one (14)</p>	15.06	311.2	247, 239, 256, 197, 183 (100%), 170	 <p>$C_{18}H_{16}O_5$ Massa: 312,32</p>	Ethanolic Crude Extract // Hexane Fraction // Ethyl acetate Fraction	Negative	Flavonoid
<p>3- [(2S,3R,4S,5R,6 R)-3,5- dihydroxy-6- (hydroxymethyl)-4- [(2S,3R,4R,5R, 6S)-3,4,5- trihydroxy-6- methyloxan-2- yl]oxyoxan-2- yl]oxy-5,7- dihydroxy-2-(4- hydroxyphenyl)</p>	9.34	593.1	593 (100%), 285, 255	 <p>$C_{27}H_{30}O_{15}$ Massa: 594,52</p>	Ethanolic Crude Extract	Negative	Glycosyla ted Flavonoid

chromen-4-one (15)							
quercetin-3-O-deoxyhexosyl(1-2)pentoside (16)	9.18	579.1	489, 475, 458, 433, 415, 384, 354, 300 (100%), 271, 255	 <p>C₂₆H₂₈O₁₅ Massa: 580,49</p>	Ethyl acetate Fraction	Negative	Glycosylated Flavonoid
PR309043 Flavanone base + 4O, 2 Prenylgancaonin E (17)	14.36	423.1	423 (100%), 381, 219, 203, 148	 <p>C₂₅H₂₈O₆ Massa: 424,49</p>	Ethanollic Crude Extract	Negative	Flavonoid

Supplementary Table 4: Results of DPPH and ABTS antioxidant assays for *M. nigra* samples

<i>Metrodorea Nigra</i>	DPPH CE ₅₀ (µg/ml)	ABTS – 414nm CE ₅₀ (µg/ml)
Rutin (pattern)	9.3506 ± 0.3997	14.3942 ± 0.1352
Ethanollic crude extract		
MnBF1	6.0759 ± 0.9013	5.1403 ± 0.1178
Fractions		
Hexane fraction	153.3310 ± 1.2086	37.7818 ± 3.5414
Dicloromethane Fraction	6.7859 ± 1.8010	2.7573 ± 0.0302
Ethyl Acetate Fraction	11.3663 ± 1.3203	7.9818 ± 0.3250
Sp1D Subfraction	12.0350 ± 0.3012	2.4190 ± 0.0653

Supplementary Table 5: Frap antioxidant assay results

<i>M. nigra</i>	Sample (µmol de Fe+2/g of sample)				
Concentration (µg/ml)	MnBF1	P1A	P1B	P1C	Sp1D
200	5432.8 ± 305.7	1786.9 ± 51.4	3200.8 ± 234.6	3670.3 ± 186.4	3818.9 ± 33.9
100	3571.7 ± 356.3	1241.1 ± 12.0	2484.2 ± 74.1	2678.6 ± 113.1	2849.4 ± 188.2
50	2381.4 ± 32.3	1042.5 ± 8.3	2049.4 ± 25.0	2055.0 ± 75.8	2175.8 ± 142.0