

Antimicrobial Activity of Flavonoids From *Piper lanceaefolium* and Other Colombian Medicinal Plants Against Antibiotic Susceptible and Resistant Strains of *Neisseria gonorrhoeae*

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Background: The successful treatment of *Neisseria gonorrhoeae* (NG) infections is increasingly problematic because of the resistance of this pathogen to multiple antimicrobial agents. This development underscores the need for new antimicrobial sources. In the current study, 21 crude methanol extracts, from 19 plants used in Colombian traditional medicine for cutaneous infections, were screened for antimicrobial activity against NG.

Methods: Extracts were screened by disc susceptibility assay. In addition, the minimum inhibitory concentrations of active compounds from *P. lanceaefolium* were assayed using a panel of 26 NG strains comprising 12 antibiotic-resistant phenotypes.

Results: In all, 71% of the crude extracts exhibited antibacterial activity against the antibiotic susceptible NG strain WHO V, whereas 10% of the extracts inhibited penicillinase-producing NG strain GC1-182. The crude extract of *Piper lanceaefolium* was the only extract to show significant activity without ultraviolet (UV) light activation. Preliminary screening identified 3 compounds in this plant possessing antimicrobial activity: the flavonoids 5,7-dihydroxyflavanone (pinocembrin), 2',4',6'-trihydroxychalcone (pinocembrin chalcone), and the prenylated benzoic acid derivative cyclolanceaefolic acid methyl ester. Pinocembrin and pinocembrin chalcone inhibited 100% of the NG panel at 64 µg/mL and 128 µg/mL, respectively, whereas cyclolanceaefolic acid methyl ester inhibited 44% of the strains at 128 µg/mL.

Conclusions: This is the first report of the antibacterial activity of Colombian plants against NG. The activity of the 2 flavonoids, pinocembrin, and pinocembrin chalcone, toward both susceptible and resistant NG strains makes them promising candidates for further research.

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The authors thank Sarah Helgeson, Sidharath Dev Thakur, and Ava Storey for technical assistance.

This paper is dedicated to the memory of Dr. G. H. N. Towers, mentor and scholar.

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DOI: 10.1097/OLQ.0b013e3181f0b0bd

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Bacterial infections continue to cause significant morbidity and mortality worldwide, often caused by treatment failure or treatment option restrictions because of the prevalence of antibiotic-resistant isolates. Resistance to antimicrobial agents may arise through many factors including inappropriate or inadequate antibiotic therapy, selective pressure, and a high prevalence of disease coupled with lack of capacity to initiate surveillance or prevention programs.¹⁻³ *Neisseria gonorrhoeae* (NG), the Gram-negative diplococcus that causes the sexually transmitted disease gonorrhea remains one of the most clinically significant and prevalent bacterial infectious diseases with an estimated worldwide incidence of >60 million cases per year.^{3,4} Untreated infections can lead to serious complications including sterility, pelvic inflammatory disease, and ectopic pregnancy, whereas also amplifying the transmission of human immunodeficiency virus in men and women.^{3,4} Effective treatment regimens are becoming increasingly limited because of the development of resistance or decreased susceptibility to the few antibiotics, which remain effective against NG, coupled with a significant lack of evidence that either new antimicrobial agents or combinations of older agents would be effective.^{1,5}

The prevalence of penicillinase producing *N. gonorrhoeae* (PPNG) and tetracycline resistant NG (TRNG) isolates, which carry plasmid-mediated resistance to penicillin or tetracycline, respectively, has precluded the use of these antibiotics for treatment for several decades.^{6,7} In addition to plasmid-mediated resistance mechanisms, gonococci may acquire resistance through chromosomal mutation at a number of different loci, possibly conferring multiple resistance to unrelated antibiotics.^{4,6} Although treatment regimens have shifted to more effective single-dose drugs such as third generation cephalosporins, fluoroquinolones, and spectinomycin in the 1990s, reduced susceptibility and de facto resistance of NG isolates to these antibiotics is now apparent.⁸ High percentages of quinolone-resistant NG first appeared in the far East and were soon reported from many areas of the world such that many jurisdictions no longer recommend these antimicrobials for treatment.⁹⁻¹² Sporadic reports of resistance to spectinomycin, coupled with its unavailability in many jurisdictions, and reduced susceptibility to ceftriaxone have also threatened the continued sustainability of these antimicrobials as therapeutic agents.^{1,8,13-15} Azithromycin is recommended for the treatment of genital infections caused by *Chlamydia trachomatis* and therefore its efficacy against coinfection with NG is of interest. Reports of reduced susceptibility and now outright resistance to this antimicrobial have also emerged.¹⁶⁻¹⁸ Given this dismaying array of resistance phenotypes, it has been questioned

TABLE 1. Ethnobotanical Information of the 21 Colombian Medicinal Plant Extracts Examined in this Study*

Extract Number	Plant Name	Common Name	Family	Part Extracted
1	<i>Eschweilera cf. rutifolia</i> S.A. Mori	Carguero	Lecythidaceae	BK
2	<i>Tagetes erecta</i> L.	Mapan	Asteraceae	FL
3	<i>Polygonum punctatum</i> Elliot	Picantillo	Polygonaceae	AE
4	<i>Iryanthera tricornis</i> Ducke	Cabo de hacha	Menispermaceae	LF
5	<i>Myrteola nummularia</i> (Poir.) O. Berg	Guayabilla	Myrtaceae	AE
6	<i>Eupatorium glutinosum</i> Lam.	Matico	Asteraceae	LF
7	<i>Piper lanceaeifolium</i> H.B. and K.	Cueche	Piperaceae	LF
8	<i>Symphonia globulifera</i> L.f.	Machare	Clusiaceae	BK
9	<i>Solanum</i> spp.	Chontara	Solanaceae	BK
10	<i>Senna reticulata</i> (Willd.)	Galves	Fabaceae	LF
11	<i>Vismia cf. macrophylla</i> Kunth	Lacre	Clusiaceae	RE
12	<i>Picrolemma sprucei</i> Hooker f.	Arbolito de casabe	Simaroubaceae	LF
13	<i>Conyza bonariensis</i> (L.) Cronq.	Tacehsajch	Asteraceae	AE
14	<i>Byrsonima verbascifolia</i> (L.) DC.	Unknown	Malpighiaceae	RB
15	<i>Byrsonima verbascifolia</i> (L.) DC.	Unknown	Malpighiaceae	LF
16	<i>Ampelozizyphus amazonicus</i> (Ducke)	Unknown	Rhamnaceae	LF
17	<i>Iryanthera megistophylla</i> A.C. Sm.	Cabo de indio	Menispermaceae	BK
18	<i>Duroia hirsuta</i> (Poepp.) K. Schum.	Unknown	Rubiaceae	LF
19	<i>Virola cf. multinervia</i> Ducke	Ambil de monte	Myristicaceae	BK
20	<i>Virola cf. multinervia</i> Ducke	Ambil de monte	Myristicaceae	RE
21	<i>Adiantum latifolium</i> Lam.	Montanero	Pteridaceae	AE

*For further information refer to López et al.²⁹

BK indicates bark; LF, leaf; RB, root bark; AE, aerial parts; RE, resin; FL, flower.

whether an era of untreatable infections has arrived, underscoring the urgency in establishing different and effective treatments for gonococcal infections.⁵

The diversity of secondary metabolites found in medicinal plants represents a valuable alternative source of potential new antimicrobial agents.¹⁹ Traditional treatments for sexually transmitted diseases are prominent in world ethnobotanies, and a few studies have identified medicinal plants showing activity against sexually transmitted organisms.^{20–28}

López et al.²⁹ collected 19 previously unstudied medicinal plants traditionally used by local healers in Colombia for treating a variety of conditions, mainly cutaneous infections, which are often bacterial in origin (Table 1). Plants were collected from 4 culturally and geographically distinct areas in Colombia: the Bajo Calima, Matavan forest, Sibundoy valley, and Middle Caqueta basin regimens. Preliminary antimicrobial screening using dried methanolic extracts in a disk diffusion assay found that the majority of these plants possessed activity against the Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus faecalis*, but not against any of the Gram-negative species then tested.²⁹ The goals of the current study were to determine whether these medicinal plant extracts had antimicrobial activity against susceptible and resistant strains of NG and to better characterize the active principals of any especially active extracts.

MATERIALS AND METHODS

Selection, Storage, and Growth of Bacterial Strains

NG reference strains WHO III, WHO V, and WHO VII (susceptible to all antibiotics) were used as controls.³⁰ WHO V and NG GC1–182, a PPNG isolate (GenBank accession No U20374.1) were selected in initial assays to test the activity of crude plant extracts. For subsequent testing of an extract's specific active components, a panel of 26 NG strains was used

representing a variety of both chromosomal and plasmid mediated antimicrobial resistance profiles. These were a collection of clinical isolates whose resistance phenotypes were determined previously using the agar dilution method and were interpreted based on the criteria of the Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS).³¹ Plasmid profiles of the 12 β -lactamase producing NG isolates were determined as previously described.³² The antimicrobial resistance phenotypes and minimum inhibitory concentration (MIC) and plasmid profiles of the NG strains are summarized in Table 2. All strains were stored at -70°C in Brain-Heart Infusion broth (Difco; Detroit, MI) +20% glycerol.

NG strains were subcultured on GC medium base agar (Difco) supplemented with 1% Kellogg's defined supplement (GCMBK agar).²⁴ PPNG and TRNG strains were grown on GCMBK agar supplemented with 5 $\mu\text{g}/\text{mL}$ of ampicillin and/or tetracycline, respectively, to ensure plasmid maintenance.⁶ Strains were incubated for 18 to 20 hours at 35°C in a humid atmosphere with 5% CO_2 . The same incubation conditions and media were used for all antimicrobial susceptibility testing. Penicillin and tetracycline were purchased from Sigma-Aldrich (St Louis, MO).

Ethnobotany of Colombian Extracts and Fractionation and Identification of *P. lanceaeifolium* Constituents

The Colombian extracts were collected and identified as described in López et al.²⁹ Briefly, fresh leaves were air-dried in the shade, preserving color and odor, then ground to a powder, and extracted with an equal volume of acetone and on a shaker for 25 minutes. The resulting extracts were filtered and concentrated to dryness (yields range from 5% to 10% of dry weight). This extract was dissolved in methanol (10 $\mu\text{g}/\text{mL}$). Isolation and identification of the various leaf phytochemical constituents of *P. lanceaeifolium* were performed using conventional phytochemical techniques as previously described.³³

TABLE 2. Antimicrobial Resistance Phenotypes of 26 *N. gonorrhoeae* Isolates

Isolate Number	Resistance Phenotype*	MICs (µg/mL)				
		PEN	TET	SPEC	CIPRO	ERY/AZI†
22 (WHO III)	Susceptible	0.063	0.125	16	0.004	0.032/ND
23 (WHO V)	Susceptible	0.5	1	16	0.008	0.125/ND
24 (WHO VII)	Susceptible	0.016	0.25	16	0.004	0.25/ND
25	Susceptible	0.5	1	32	0.004	2/ND
26	Susceptible	0.5	0.5	32	0.004	1/ND
27	Susceptible	<0.008	0.25	32	0.004	0.5/ND
1	TRNG	0.25	32	32	0.004	1/ND†
3	TRNG	0.25	16	32	0.004	1/ND
2	TRNG/ERY ^R	0.125	16	32	0.004	2/ND
4	PPNG‡	128	1	32	0.004	0.125/ND
5	PPNG	16	0.5	32	0.004	0.5/ND
6	PPNG	8	0.5	32	0.004	0.5/ND
8	PPNG	16	1	32	0.004	0.5/ND
11	PP/TRNG	8	16	16	0.004	0.5/ND
20	PPNG/ERY ^R	32	1	32	0.008	2/ND
21 (GC1-182)	PPNG/CMTR/ERY ^R	128	4	16	0.004	2/0.5
16	PPNG/CMTR/ERY ^R	128	2	32	0.008	4/ND
17	PPNG/CMTR/ERY ^R	64	2	32	0.016	4/ND
19	PPNG/CMTR/ERY ^R /SPEC ^R	64	2	>256	0.008	4/ND
28	PPNG/CMTR/CIPRO ^R	16	2	16	2	0.25/ND
30	PPNG/CMTR/ERY ^R /AZI ^R	>128	2	32	0.032	8/1
12	CMRNG/ERY ^R	2	2	32	0.016	4/ND
13	CMRNG/ERY ^R	2	4	32	0.032	8/ND
29	CMRNG/CIPRO ^R	2	4	16	2	0.25/ND
15	CMTR	0.016	2	32	0.004	0.25/0.25
14	CMTR/SPEC ^R /AZI ^R	0.5	4	>256	0.016	1/1

*TRNG: TET MIC ≥16 µg/mL and non-PPNG; PPNG: penicillinase-producing NG and non-TRNG; CMTR: TET MIC ≥2 and <16 µg/mL; CMRNG: chromosomally mediated resistance NG, TET MIC ≥2 and <16 µg/mL, PEN MIC ≥2 µg/mL, abd non-penicillinase producing; CIPRO^R: ciprofloxacin MIC ≥1 µg/mL; SPEC^R: spectinomycin MIC ≥128 µg/mL; AZI^R: azithromycin MIC >1 µg/mL; ERY^R: erythromycin MIC ≥2 µg/mL.

†ND indicates not determined.

‡Plasmid profiles of the 12 PPNG strains were determined as previously described.³² All PPNG carried Africa (3.2 MDa) type plasmids except for Isolate numbers 4 and 21 which carried Asia (4.5 MDa) type plasmids.

Disk Diffusion Assays

Crude extracts were dissolved in methanol (MeOH) and placed on blank (6 mm) paper discs for a final concentration of 2 µg extract/disc. The solvent was allowed to evaporate and the extract-laden discs were stored in the dark at -70°C until use. Inocula were prepared in accordance with National Committee for Clinical Laboratory Standards guidelines.³⁴ Direct colony

suspensions of overnight subcultures were diluted in Mueller Hinton (MH) broth (Difco) and adjusted to a 0.5 McFarland turbidity standard (approximately 10⁸ Colony Forming Units [CFU]/mL). GCMBK plates were then inoculated by streaking this suspension with a sterile cotton swab over the entire plate, which was twice rotated 90 degrees to evenly spread the inocula. The extract-treated disc was placed in the centre of an

TABLE 3. Disk Susceptibility Assays of Methanol Plant Extracts Against *N. gonorrhoeae* WHO V and GC1-182

Strain	UV Exposure Time (min)	Zone of Inhibition for Plant Extracts (Extract Number)*		
		<10 mm	10-20 mm	>20 mm†
WHO V	30		13, 15, 17, 18, 20, 21	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 19
WHO V	20	18, 21	1, 4, 5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 19, 20	2, 3, 7, 9, 10
WHO V‡	0	16, 17, 18, 20	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 19	7
GC1-182	20	4, 6, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21	1, 3, 7, 8, 15	2, 5

*Extract number (Table 1).

†Zone of inhibition (ZOI) of >20 mm indicates active extract.

‡Extracts 4 and 21 were not tested (n = 19).

inoculated plate, and the plate was then placed under 4 blacklite blue 20 W fluorescent near ultraviolet (UV) tubes (emitting between 320 and 400 nm at an intensity of 10 w/m²) for 0, 20, or 30 minutes, exposure times that were previously found to be nonlethal to NG (unpublished data). This was performed to determine whether exposure to near UV light would have any effect on the extract's activity, as many phytochemicals require such exposure to elicit biologic activity in nature.³⁵ Plates were then incubated at 35°C in a humid environment supplied with 5% CO₂. Plates were observed normally at 20 hours and the observation extended to 42 and 66 hours if needed. Results were recorded as the average diameter (including the disc), in mm, of the zone of inhibition (ZOI), which comprised the average of 3 measurements taken at 60 degree angles to each other. Based on the various zones of inhibition observed, plant extracts that created a ZOI of at least 20 mm after ≤20 minutes of UV exposure and 18 hours of incubation were considered to be very active against NG.

MIC Testing

The agar dilution method was used to better quantify the activity of isolated compounds in identified active crude extracts.³¹ Throughout all such testing, the concentration of MeOH did not exceed 2% of the total agar volume, a concentration previously found to be nontoxic to representative NG strains (data not shown). Because of the small amount of available material, a small scale preliminary assay was employed using 24 well microplates (Corning Costar, Acton, MA) to test 10 isolated fractions of *P. lanceaeifolium* against NG. The fractions were dissolved in 50% MeOH, diluted, and mixed with molten GCMBK agar for a final concentration range in the plate wells of 0.25 to 256 μg/mL (1 mL aliquots). Bacterial suspensions of NG strains WHO V, WHO VII, and GC1-182 were prepared in MH broth and then diluted for an inoculum concentration of approximately 10⁵ CFU/mL in the microplate of which a 10 μL inoculum was delivered to the agar plates. Plates were then incubated at standard conditions and inspected visually the following day for MIC determinations.

Any fractions showing MICs <256 μg/mL were selected for more extensive testing against the panel of 26 NG strains using the method described above, except that the volumes were scaled up to prepare the fractions in standard petri dishes. Fractions were dissolved in 50% MeOH and serially diluted to give a final concentration range of 0.25 to 128 μg/mL (higher concentrations could not be tested because of lack of material). Bacterial suspensions were prepared in MH broth, standardized to a 0.5 McFarland turbidity standard, and diluted to an inoculum size of approximately 10⁵ CFU/mL; plates were inoculated using a Steers replicator which delivered 10 μL. After inoculation, the plates were air-dried (15–20 minutes) before inversion and incubation. After 18 hours incubation at standard conditions, plates were examined visually and MICs recorded. Compounds were tested in duplicate 2 times and penicillin controls (0.063–128 μg/mL) were included with all assays. MICs were determined by visual inspection and recorded as the lowest concentration at which no growth was visible.

RESULTS

Colombian Plant Crude Extracts

Those extracts considered to be very active in inhibiting NG strain WHO V (Table 3) included extracts 2 (*Tagetes*

erecta), 3 (*Polygonum punctatum*), 7 (*Piper lanceaeifolium*), 9 (*Solanum* sp.), and 10 (*Senna reticulata*). These extracts comprised 24% of the extracts tested. After 30 minutes of UV exposure, 71% of the extracts exhibited significant activity whereas only 1 extract (*P. lanceaeifolium*) exhibited activity with no UV exposure. Thus, the length of UV exposure had a clear effect on the antimicrobial activity of the plant extracts against NG WHO V. Although 74% of the extracts had some activity without UV exposure, it was considerably weaker (intermediate ZOI sizes of 10–20 mm) than the activities of the extracts following UV exposure. A weak trend toward reduction in ZOI sizes (by 1–3 mm) over the 66 hour incubation period was noticed among most extracts, regardless of the initial UV exposure time (data not shown). This appeared to be the result of colony regrowth within the periphery of the ZOI.

Because of a shortage of plant material, the NG strain GC1-182, a PPNG and chromosomally-mediated resistance NG strain (GMRNG), was tested at 1 UV timepoint (20 minutes). Fewer extracts inhibited the growth of GC1-182 (10%)

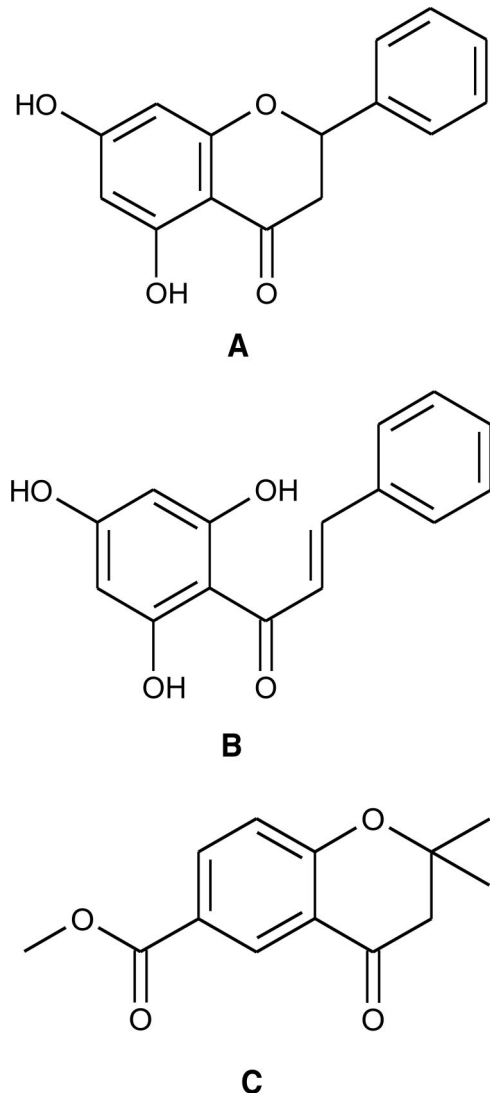


Figure 1. A, Structures of pinocembrin; B, pinocembrin chalcone; and C, cyclolanceaeolic acid methyl ester.

TABLE 4. Cumulative Percent Inhibition by Pinocembrin, Pinocembrin Chalcone, and Cyclolanceaefolic Acid Methyl Ester

Resistance Phenotype* of <i>N. gonorrhoeae</i> Isolates	n	Cumulative % Inhibition [†]																
		Pinocembrin					Pinocembrin Chalcone					Cyclolanceaefolic Acid Methyl Ester						
		≤2	4	8	16	32	64	4	8	16	32	64	128	8	16	32	64	≥128
Susceptible	6	0	0	0	17	50	100	0	0	33	33	67	100	0	0	17	17	33
TRNG or TRNG/ERY ^R	3	0	0	0	0	66	100	0	0	0	33	100	100	0	0	0	33	67
PPNG	4	25	25	25	25	100	100	0	25	25	75	100	100	0	0	0	0	50
PP/TRNG	1	0	0	100	100	100	100	0	100	100	100	100	100	0	100	100	100	100
PPNG/ERY ^R	1	0	0	0	0	100	100	0	0	0	100	100	100	0	0	0	100	100
PPNG/CMTR/ERY ^R	3	0	0	0	0	66	100	0	0	0	33	66	100	0	0	0	0	66
PPNG/CMTR/ERY ^R /SPEC ^R	1	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	100
PPNG/CMTR/CIPRO ^R	1	0	0	0	0	100	100	0	0	0	0	100	100	0	0	0	0	0
PPNG/CMTR/ERY ^R /AZI ^R	1	0	0	0	0	100	100	0	0	0	100	100	100	0	0	0	100	100
CMRNG/ERY ^R	2	0	0	0	0	50	100	0	0	0	0	50	100	0	0	0	0	0
CMRNG/CIPRO ^R	1	0	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0
CMTR	1	0	0	0	0	100	100	0	0	0	100	100	100	0	0	0	0	0
CMTR/SPEC ^R /AZI ^R	1	0	0	0	0	100	100	0	0	0	100	100	100	0	0	0	0	100
Total (%)	26	4	4	8	12	73	100	0	8	15	46	81	100	0	4	8	19	50

*see Table 2.

[†]Percentages rounded to the nearest whole number and represent the average inhibition at each MIC from 2 independent experiments. Concentrations of the extracts were indicated in bold ($\mu\text{g}/\text{mL}$).

than WHO V (24%), with only extract 2 (*T. erecta*) inhibiting both strains. Interestingly, extract 5 (*Myrteola nummularia*) demonstrated higher activity at 20 minutes against GC1–182 than at 30 minutes UV exposure against WHO V. The zones of inhibition were also generally smaller for GC1–182 than WHO V (average of 11 mm and 17 mm, respectively).

Active Constituents of Extract 7 (*Piper lanceaefolium*)

Based on the activity of extract 7 in the absence of UV light, we examined 10 fractions of *P. lanceaefolium* leaf preparations to determine which components were responsible for this crude extract's antimicrobial activity. In the preliminary assay, 2 of the 10 fractions showed moderate activity (MICs of 16–32 $\mu\text{g}/\text{mL}$) against NG strains WHO V, WHO VII, and GC1–182, whereas a third fraction showed minimal activity against WHO VII (data not shown). The remaining 7 fractions demonstrated MICs of >256 $\mu\text{g}/\text{mL}$ against the 3 strains. Three active fractions were selected for further testing, which are as follows: the flavanone pinocembrin 5,7-dihydroxyflavanone; its chalcone, pinocembrin chalcone (2',4',6'-trihydroxychalcone); and, cyclolanceaefolic acid methyl ester (a prenylated benzoic acid derivative) (Fig. 1).^{33,36,37}

Pinocembrin was the most active constituent with MICs of 64 $\mu\text{g}/\text{mL}$ being inhibitory to 100% of the panel of NG strains (Table 4). Most of the PPNG strains had MICs to pinocembrin of 32 $\mu\text{g}/\text{mL}$ or less. Pinocembrin chalcone was similarly effective against the entire panel of NG strains at the 2-fold higher concentration of 128 $\mu\text{g}/\text{mL}$ (Table 4). Cyclolanceaefolic acid methyl ester was less active, inhibiting the growth of <50% of the strains tested at the highest concentration of 128 $\mu\text{g}/\text{mL}$ (Table 4).

DISCUSSION

We show that 24% of the 21 Colombian plant extracts were significantly active (ZOI of ≥ 20 mm) against NG after

20 minutes of UV exposure, whereas with longer UV exposure times all the extracts showed some activity. Because UV exposure had a clear effect on the extract's effectiveness, the decrease in ZOI size associated with increasing the incubation time could be due to the extended period in the dark incubator. The regrowth of colonies after 66 hours suggests that some crude extracts were demonstrating a bacteriostatic effect.

Although the extracts tested had been previously found to be active against only Gram-positive bacteria, our findings of activity against a previously untested Gram-negative genus suggest a broader spectrum activity for these extracts.²⁹ Of the plant extracts found to be significantly active against NG, only *P. lanceaefolium*, *T. erecta*, and *P. punctatum* have previously been identified as possessing antimicrobial activity.^{29,38,39} Interestingly, *T. erecta* was also 1 of only 2 plant extracts (along with *M. nummularia*) to demonstrate significant activity against the PPNG/CMRNG strain GC1–182.

Antimicrobial constituents from *Iryanthera megistophylla*, *Symphonia globulifera*, and *Eupatorium glutinosum*, plants that exhibited varying levels of activity against NG (depending on UV exposure times), have also been identified.^{40–43} Constituents from these plants active against other bacteria might also be implicated in the activity of these plants against NG isolates. For example, iryantherin K and procyanidin B-2 (from root bark ethanol extracts of *I. megistophylla*) showed MICs of 50 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ respectively against *S. aureus*,⁴¹ whereas Nkengfack et al.⁴² found high activity against *S. aureus* and *B. subtilis* (MICs of 3–14 $\mu\text{g}/\text{mL}$) among 3 prenylated xanthenes from the root bark of *S. globulifera*.

We have also shown that pinocembrin and pinocembrin chalcone from *P. lanceaefolium* possess moderate antimicrobial activity against both susceptible and resistant strains of NG. Pinocembrin has been previously identified as possessing antimicrobial activity.^{36,44} Using a quantitative structure-activity relationship (QSAR) approach, Alcaráz et al.³⁶ found that

chalcones (MICs of 16–64 $\mu\text{g/mL}$) generally have more activity than flavanones against methicillin-resistant *S. aureus* strains. Pinoembrin was reported as the most active flavanone tested with an MIC of 128 $\mu\text{g/mL}$, consistent with results we obtained assaying pinoembrin against 2 clinical methicillin-resistant *S. aureus* strains (MICs of 128 $\mu\text{g/mL}$, data not shown). Pinoembrin chalcone has been identified as an antimicrobial compound from *Helichrysum trilineatum*,³⁷ whereas cyclolanceaefolic acid methyl ester and similar prenylated benzoic acid derivatives are known to have antifungal activity.³³

Our results suggest that further investigation into the activity of pinoembrin and pinoembrin chalcone is warranted, especially because their activity against strains of NG with different antimicrobial resistance phenotypes suggests a broad, but potentially novel, antimicrobial mechanism. The investigation of synergistic interactions between these flavonoids and currently used antibiotics, which could minimize the pharmaceutical drug load and risk of resistance among patients, as well as reducing treatment costs, might also provide attractive therapeutic alternatives for treating gonococcal infections.

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