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Antimicrobial compounds isolated from *Haematoxylon brasiletto*

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ABSTRACT

Ethnopharmacological relevance: The decoction of the bark of *Haematoxylon brasiletto* Karst. (Leguminosae), commonly known as "Palo Brasil", is used in the Mexican traditional medicine to treat mouth and kidney infections, hypertension, stomach upsets, gastric ulcers and diabetes.
Aim of the study: The present study was performed to evaluate the antimicrobial effects of the methanolic extract of the bark of *Haematoxylon brasiletto*.
Materials and methods: A panel of 12 bacteria and the yeast *Candida albicans* were used. Minimum inhibitory concentrations (MICs) were determined by the standard broth microdilution method.
Results: The results indicate that the extract of *Haematoxylon brasiletto* inhibited the growth of eight of the tested microorganisms at a concentration limit of 128 μg/mL. For the tested compounds the MIC values ranged from 8.7 to 128 μg/mL.
Conclusions: The overall results provide promising baseline information for the potential use of the extracts from *Haematoxylon brasiletto* as well as some of the isolated compounds in the treatment of bacterial infections.

1. Introduction

Haematoxylon brasiletto Karst. (Leguminosae) is a large tree very abundant in the south-east Mexico. It is commonly called "azulillo", "corteza de Brasil", "palo Brasil", "palo de tinta" and "palo tinto". The wood derives its name from the word *baza*, meaning fiery red, and has no geographic connection with Brazil or with common brazilwood, *Caesalpinia echinata* or *Caesalpinia brasilensis* (Craig et al., 1965). As a home remedy, the bark prepared as a tea is employed for treating hypertension, stomach upsets, mouth infections, diarrhea, gastric ulcers and diabetes (Argueta et al., 1994). An ethanolic extract of the stem bark of *Haematoxylon brasiletto* was found to inhibit the growth of *Escherichia coli* O157:H7 (EHEC), verotoxin production, and adhesion of *Escherichia coli* O157:H7 to HeLa cells (Heredia et al., 2005). In previous work, the bark has afforded hematoxylin (**1**) and brazilin (**2**) (Sanchez-Marroquin et al., 1958; Pratt and Yuzuriha, 1959). The plant was selected for fractionation after its methanol-soluble extract exhibited significant antibacterial activity against 12 bacteria and the yeast *Candida albicans*.

2. Methodology

2.1. Plant material

The air-dried bark of the *Haematoxylon brasiletto* Karst. were obtained in the Mercado de Sonora, México, D.F. Voucher specimens (Bye 20339) have been deposited at the ethnobotanical collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

2.2. Activity guided compound isolation

The air-dried stem bark (1.0 kg) of *Haematoxylon brasiletto* was extracted by maceration with methanol (3× 2 L) at room temperature (24 h each) and the combined MeOH extracts were evaporated under reduced pressure, yielding a residue (100 g), which was partitioned with petroleum ether, dichloromethane and ethyl acetate. Water was added to the MeOH extract to afford a 50% aqueous MeOH solution before partitioning with dichloromethane. The resulting four extracts (petroleum ether, dichloromethane, ethyl acetate and aqueous) were evaluated against 12 bacteria and the yeast *Candida albicans*. The crude ethyl acetate-soluble extract (35.7 g) was found to exhibit the stronger antibacterial activity (Table 1). Hence the ethyl acetate-soluble extract was subjected to silica gel column chromatography and eluted with gradient mixtures of hexanes–dichloromethane and then acetone and acetone–methanol of increasing polarity to give 15 pooled

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Table 1
Minimum inhibitory concentrations in of the extract (μg/mL), the compounds isolated from the bark of *Haematoxylon brasiletto* bark and reference antibiotics (μg/mL and mM)

Microorganisms	Extract	Compounds								Reference antibiotics	
	MeOH	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	PG[a]	CHX[a]
Staphylococcus aureus 375[b]	64	16 (4.83)	16 (4.57)	32 (5.76)	32 (5.88)	32 (5.44)	32 (4.03)	128 (20.99)	64 (13.82)	0.06 (0.02)	
Staphylococcus aureus 310 (MR)[b]	128	32 (9.86)	32 (9.15)	64 (11.52)	64 (11.76)	128 (21.76)	128 (16.13)	128 (20.99)	128 (27.64)	64 (21.40)	nt
Staphylococcus aureus ATCC 25923[b]	16	16 (4.83)	16 (4.57)	32 (5.76)	16 (2.94)	32 (5.44)	64 (8.06)	64 (10.49)	32 (6.91)	0.06 (0.02)	nt
Enterococcus faecium 379 (VR)[b]	16	16 (4.83)	16 (4.57)	32 (5.76)	16 (2.94)	32 (5.44)	32 (4.03)	64 (10.49)	128 (27.64)	32 (10.70)	nt
Bacillus subtilis 327[b]	16	32 (9.66)	64 (18.30)	64 (11.52)	64 (11.76)	64 (10.88)	64 (80.6)	64 (10.49)	>128 (27.64)	32 (10.70)	nt
Escherichia coli ipm 389[b]	>128	64 (19.32)	128 (36.61)	>128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	2 (0.66)	nt
Escherichia coli 442[b]	>128	64 (19.32)	64 (36.61)	>128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	32 (10.7)	nt
Escherichia coli ATCC 25922[b]	>128	128 (38.64)	128 (36.61)	128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	128 (42.80)	nt
Klebsiella pneumonia 425[b]	128	128 (38.64)	128 (36.61)	>128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	128 (42.80)	nt
Pseudomonas aeruginosa 339[b]	>128	128 (38.64)	>128 (36.61)	128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	>128 (42.80)	nt
Candida albicans 54[b]	>128	>128 (38.64)	>128 (36.61)	>128 (23.04)	>128 (23.52)	>128 (21.76)	>128 (16.13)	>128 (20.99)	>128 (27.64)	>128 (42.80)	nt
Streptococcus mutans[c]	36.3	17.8 (5.47)	13.5 (3.83)	14.8 (2.66)	20.6 (3.79)	76.8 (13.05)	18.9 (2.38)	23.6 (3.87)	52.4 (11.32)	nt	1.7 (0.85)
Porphyromonas gingivalis[c]	19.5	13.5 (4.07)	8.7 (2.48)	17.8 (1.56)	17.8 (3.27)	60.7 (10.32)	17.0 (2.14)	17.8 (2.92)	44.7 (9.65)	nt	0.6 (0.30)

Hematoxylin (**1**), brazilin (**2**), caffeic acid (**3**), methyl gallate (**4**), gallic acid (**5**), phloroglucinol (**6**), 4-hydroxycinnamic acid (**7**), and 5-methoxypsoralen (**8**).

[a] Reference antibiotics—PG: penicillin; CHX: chlorhexidine gluconate.

[b] Standard broth microdilution.

[c] *In vitro* growth inhibition assay performed in 96-well microtiter plates.