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THE FLAVONOL CALYCOPTERIN FROM THE ANTIMICROBIAL ETHYL ACETATE EXTRACT OF *Marcetia latifolia*

T. C. C. Leite,[1] S. T. Martinez,[2] A. P. T. Uetanabaro,[3] T. R. S. Silva,[4] and A. Branco[1]*

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In Brazil, the Melastomataceae family is the sixth most represented category of angiosperm plants present in territorial flora, with 65 genera and 1500 species identified. In this context, the genus *Marcetia* contains 28 species that are endemic to the Brazilian Campos Rupestres located between the states of Minas Gerais, Goias, and Bahia [1]. To the best of our knowledge, there are no published studies on the biological activity and chemical composition of *Marcetia* species.

Here, the antimicrobial activities of extracts of *Marcetia latifolia* have been investigated using the disc diffusion method. A total of ten microorganisms, seven bacteria and three yeasts, were tested. The results are summarized in Table 1. The hexane extract showed activity against two bacteria and one yeast. However, the EtOAc extract showed good antimicrobial activity on two *Candida* species and on all of the different strains of *Staphylococcus aureus*, a Gram-positive bacteria. None of the extracts presented activity against the tested Gram-negative bacteria. The lower sensitivity of Gram-negative bacteria has been explained by the presence of an outer membrane surrounding their cell wall, which restricts the diffusion of hydrophobic compounds through their lipopolysaccharide covering. This would explain why hexane and EtOAc extracts were effective on the tested Gram-positive cultures, corroborating other publications [2].

Because of the significant inhibition of the EtOAc extract from *M. latifolia*, it was fractionated by open-column chromatography using silica gel to furnish **1a–d** and **2**. The alkanes **1a–d** were characterized by GC-MS analysis [3], and the chemical structure of **2** as 5,4′-hydroxy-3,6,7,8-tetramethoxyflavone (calycopterin) was determined using spectroscopic analyses, including UV, MS, and ^{1}H and ^{13}C NMR [4].

Thus, this is the first report on the biological activity and isolation of natural products from *Marcetia*. The homologous series of alkanes contained between 23 to 29 carbons [3], and non-glycosylated flavonoids [5] are characteristics of the species of Melastomataceae. However, flavonoids with complete oxygenation at the A ring are rare in this family at the present moment, except in compound **2** described in this work and in *Mouriri pusa*, which bioproduces 6,8-dihydroxy-kaempferol-3-*O*-β-D-glucopyranoside [6].

Plant Material. *Marcetia latifolia* specimens were collected in Morro-do-Chapeu (Chapada Diamantina-BA), Bahia, Brazil, in April 2006, and were identified by Dr. Tania Regina dos Santos Silva. A voucher specimen (No. 119.472) has been deposited at the Herbarium of the Department of Biology, State University of Feira de Santana (HUEFS).

Obtainment of the Extracts. Air-dried and powdered aerial parts (977 g) of *M. latifolia* were successively macerated with hexane (2 L), EtOAc (2 L), and MeOH (2 L) at room temperature. The extracts were concentrated under reduced pressure to give the crude hexane (42.4 g), EtOAc (47.3 g), and MeOH (53.2 g) extracts.

Antimicrobial Activity. The antibacterial activity of the fresh plant residue was tested using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) [7], with some modifications. The microbial cultures tested were *Escherichia coli* CCMB 258; *E. coli* CCMB 261, which is sensitive to trimethoprim and resistant to sulfonamide; *Pseudomonas aeruginosa* CCMB 268; *Salmonella choleraesius* CCMB 281; *Staphylococcus aureus* CCMB 262, which is resistant to streptomycin and dihydrostreptomycin; *S. aureus* CCMB 263; *S. aureus* CCMB 264, which is resistant to novobiocin; *Candida albicans* CCMB 266; *C. albicans* CCMB 286, which is resistant to fluconazole and amphotericin B; and *C. parapsilosis* CCMB 288, which is resistant to fluconazole and amphotericin B.

1) Departamento de Saude, Universidade Estadual de Feira de Santana, 44036-900, Feira de Santana, Brazil, fax: +55 75 32248159, e-mail: branco@uefs.br; 2) Instituto de Quimica, Universidade Federal do Rio de Janeiro, 32045-000, Rio de Janeiro, Brazil; 3) Departamento de CienciasBiologicas, Universidade Estadual de Santa Cruz, 45662-900, Ilheus, Brazil; 4) Departamento de Biologia, Universidade Estadual de Feira de Santana, 44036-900, Feira de Santana, Brazil. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 427–428. Original article submitted January 27, 2011.

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TABLE 1. Antimicrobial Activity of the Extracts of *Marcetia latifolia*

Microorganism	Extract		C+
	hexane	ethyl acetate	
Bacteria			
Gram-negative			
E. coli CCMB 258	–	–	15.48 ± 1.58
E. coli CCMB 261	–	–	24.13 ± 0.70
P. aeruginosa CCMB 268	–	–	9.47 ± 0.49
S. choleraesius CCMB 281	–	–	10.05 ± 1.38
Gram-positive			
S. aureus CCMB 262	9.00 ± 0.00	11.00 ± 2.00	27.46 ± 0.97
S. aureus CCMB 263	–	12.08 ± 0.14	31.55 ± 1.56
S. aureus CCMB 264	17.85 ± 0.29	11.45 ± 1.94	34.50 ± 1.44
Yeasts			
C. albicans CCMB 266	–	9.42 ± 0.14	20.09 ± 1.40
C. albicans CCMB 286	–	–	23.50 ± 1.34
C. parapsilosis CCMB 288	11.50 ± 0.50	13.83 ± 1.15	8.83 ± 0.49

Methanolic extract – without inhibition; –: without inhibition; C+: positive controls (erythromycin for bacteria and nystatin for yeast).

The cultures were grown on Mueller Hinton Agar (MHA) media (pH 7.4 ± 2) at 37°C/24 h for bacteria and at 28°C/48 h for yeasts. Sterilized filter paper disks of 6 mm were impregnated with 5 μL of the sterilized extracts (100 mg/mL) on 0.22 μm membranes and then poured onto MHA media previously sown with 100 μL of the test microorganism (1.5×10^7 UFC for bacteria and 1.5×10^4 UFC for yeasts). The disks were also impregnated with 5 μL of erythromycin (10 mg/mL) (a macrolide antibiotic), 5 μL of nystatin (20 mg/mL) (a polyene antifungal drug), 5 μL of hexane, 5 μL of EtOAc, and 5 μL of MeOH. The plates were incubated under the same conditions described above. After the incubation period, the inhibition area was measured. The tests were conducted in triplicate.

Fractionation of EtOAc Extract and Isolation of Natural Compounds. The active crude EtOAc extract (40 g) was chromatographed on a column packed with 90 g of silica gel (0.04–0.063 mm) to furnish the following fractions: 1 (hexane, 200 mL), 2 (hexane–EtOAc 1:1, 200 mL), 3 (EtOAc, 200 mL), 4 (EtOAc–MeOH 1:1, 200 mL), and 5 (MeOH, 22 mL). The fractions were monitored by TLC. Fractions 1 and 2 were combined and analyzed by GC-MS, which showed four peaks in the respective total ion chromatogram. The fragmentation patterns in the mass spectra of these peaks made it possible to identify the alkanes **1a–1d**.

Fraction 4 (47.3 g) was chromatographed on a column packed with silica gel (0.04–0.063 mm) in the order of crescent polarities to produce yellow crystals (**2**, 19 mg).

Alkanes Mixture. White amorphous solid (in MeOH), pentacosane (*m/z* 380 $[M]^{\cdot +}$, $C_{25}H_{52}$, **1a**), heptacosane (*m/z* 408 $[M]^+$, $C_{27}H_{56}$, **1b**), nonacosane (*m/z* 436 $[M]^+$, $C_{29}H_{60}$, **1c**), and hentriacontane (*m/z* 464 $[M]^+$, $C_{31}H_{64}$, **1d**).

Calycopterin (2): mp 223–225°C (MeOH). UV (MeOH, λ_{max}, nm): 204, 233, 277, 336; + $AlCl_3$: 209, 238, 288, 311, 362; + $AlCl_3$ + HCl: 204, 285, 310, 358. PMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.40 (1H, s, HO-5), 7.96 (2H, d, J = 8.19, H-2′, 6′), 6.96 (2H, d, J = 8.19, H-3′, 5′), 4.01 (3H, s, 3-OCH_3), 3.86 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.76 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 155.9 (C-2), 137.5 (C-3), 178.3 (C-4), 148.1 (C-5), 135.0 (C-6), 151.9 (C-7), 131.9 (C-8), 144.0 (C-9), 106.5 (C-10), 129.5 (C-2′), 115.8 (C-3′), 160.0 (C-4′), 129.5 (C-5′), 115.8 (C-6′), 59.1 (3-OCH_3), 60.2 (6-OCH_3), 61.3 (7-OCH_3), 60.8 (8-OCH_3). MS-EI (70 eV) *m/z* (I_{rel}, %): 374 (M^+, 70), 359 (100), 329 (6), 226 (A_1, 1), 211 ($A_1 - CH_3$, 12), 209 (A_1 – OH, 25), 183 ($A_1 - CH_3$ – CO, 13), 178 (7).

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