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Antifungal activities of rotenoids from seeds and roots of *Clitoria fairchildiana*

Atividade antifúngica dos rotenoides das sementes e raiz de *Clitoria fairchildiana*

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Abstract

Clitoria fairchildiana (synonym *Clitoria racemosa*) is a tree belonging to the Leguminosae family growing in several Brazilian regions and it has in its composition rotenoids with unusual structures. The aim of this work is to determinate the antimicrobial activity rotenoids from *C. fairchildiana*. Clitoriacetal, 6-desoxyclitoriacetal, stemonal and stemonone were isolated from the roots and 11-desoxyclitoriacetal from seeds by different chromatographic techniques and identified by spectrometric data analyzes. The antimicrobial activity was obtained using different culture media and the results confirm the importance of the junction of the ring B/C and the pattern of hydroxylation of these compounds in antifungal activities. This is the first time antimicrobial activities of these rotenoids were determined.

Keywords: *Clitoria fairchildiana*. Rotenoids. Antifungal activities.

Resumo

Clitoria fairchildiana (sinônimo *Clitoria racemosa*) é uma árvore da família Leguminosa encontrada em várias regiões brasileiras e possui na sua composição rotenoides de estruturas não usuais. O objetivo do presente trabalho é determinar a atividade antimicrobiana de cinco rotenoides isolados das raízes e sementes da *C. fairchildiana*. Clitoriacetal, 6-desoxiclitoriacetal, stemonal e stemonona foram isolados das raízes e o 11-desoxiclitoriacetal isolado das sementes por meio de diferentes técnicas cromatográficas e identificados através da análise de dados espectrométricos. A atividade antimicrobiana foi obtida utilizando diferentes meios de cultura e os resultados confirmam a importância da junção do anel B/C e o padrão de hidroxilação dos rotenoides na atividade antifúngica. Este é o primeiro relato de atividades antimicrobianas de rotenoides de *Clitoria*.

Palavras-chave: *Clitoria fairchildiana*. Rotenoides. Atividade antifúngica.

Introduction

Clitoria fairchildiana R. A. Howard (Fabaceae), synonym of *Clitoria racemosa* Benth, is a Brazilian native tree also found in tropical regions of South America and Caribe[1]. It is popularly known as *sombreiro*, *faveira* or *palheteira* and it is characterized by being a leafy species, with very attractive violet flowers and so is widely used in arborization of parks, gardens and roads in Brazil[2]. Plants of this genus are frequently employed in the folk medicine. *Clitoria macrophylla* has been used against skin diseases and *Clitoria ternatea* infusion has been employed as anti-inflammatory and also for lung infections[3-5].

In literature, there are studies of anti-inflammatory, cytotoxicity, alelophatic, insecticide and antioxidant ativities of *C. fairchildiana* extracts[6-10]. Previous chemical studies dealing with this plant isolated rotenoids as the main compounds present in the organic extracts and some of them showed cytotoxic and anti-inflammatory activities[11-16]. The rotenoids occurring in this genus are unusual since they do not present the E-ring [6, 17-20]. In this way, this work describes the isolation and the antimicrobial activities of rotenoids isolated from *C. fairchildiana* roots and seeds extracts, and a correlation between their structures and observed activity was also checked.

Materials and methods

Plant Material

Roots and seeds of *C. fairchildiana* were collected from different tree specimens surrounding Campus of Universidade Federal da Bahia, in Salvador (BA), Brazil, in April 2010. The plant was identified by Prof. Maria Lenise S. Guedes and a voucher was deposited in Herbarium Alexandre Leal Costa, Instituto de Biologia, UFBA, under #70124.

Extraction and the rotenoid isolation

The roots (25g) and seeds (246g) of *C. fairchildiana* were dried and powdered and sequentially they are submitted to maceration at room temperature with methanol (MeOH). The obtained MeOH extracts (8.7g from roots and 21.25g from seeds) were submitted to evaporation under vacuum and then partitioned between $CHCl_3$ and MeOH:H_2O (4:1) furnishing the $CHCl_3$ soluble fractions of the extracts.

The rotenoids clitoriacetal[1], 6-desoxiclitoriacetal[2], stemonal[3] and stemonone[4] were isolated from the roots employing previously described methodology [6]. Briefly, the root extract soluble in $CHCl_3$ (5.3 g) was submitted to Column Chromatography (CC) employing silica gel 60 (0.063-0.200 mm, Acros) and eluted with mixtures of $CHCl_3$: MeOH. The fractions (50 mL each) eluted with $CHCl_3$: MeOH (9:1) and were monitored by Si 60 Thin Layer Chromatography (TLC) (F254, Fluka) in a UV cabinet. These procedures permitted to obtain 6-deoxyclitoriacetal[2] and clitoriacetal[1]. In addition, the fractions eluted with $CHCl_3$:MeOH (4:1) was submitted to a Preparative TLC (PTLC) and eluted twice with $CHCl_3$:MeOH (9:1) furnishing the rotenoids stemonal[3] and stemonone[4] .

The compound 11-desoxyclitoriacetal[5] was isolated from the $CHCl_3$ seeds extract. From 4.94 g of this extract that was subject to a silica gel 60 CC and eluted with mixtures of $CHCl_3$:MeOH. This procedure permitted to obtain 0.0300g of 11-desoxyclitoriacetal[5,11]. This procedure was monitored by TLC plates employing solutions of $AlCl_3$ as revelator.

Evaluation of the antimicrobial activities

The microorganisms employed in all the tests were maintained in Lignieri[21] medium at 5ºC. A day before the tests the antimicrobial activities of *Staphylococcus aureus* (CBAM324), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (WT), *Escherichia coli* (CBAM 002), *Enterobacter aerogenes* (ATCC 13048), *Proteus mirabilis* (ATCC 15200), *Pseudomonas aeruginosa* (ATCC 25003), *Candida albicans, Candida glabrata, Candida kruzei*, and *Candida parapsilosis* were plated in Petri dishes containing Mueller Hinton medium and incubated at 36°C for bacteriological oven for 24 hours. At the end of incubation, plates were removed, and the colonies used to prepare the microbial suspension.

After the activation, one or two colonies of the specific microorganism were removed from the Petri dish employing a platinum wire loop and inoculated in a glass tube containing 2mL of sterilized Mueller Hinton broth. This procedure was performed for all bacteria and yeasts used. The tube was homogenized, and turbidity was measured and compared to the MacFarland scale[21]. In sequence, the tubes were incubated at 36ºC for 1 hour.

After the incubation period, the tubes were homogenized, and turbidity compared with the tube 1 on the scale. If the turbidity obtained after incubation were higher than that of the tube 1 of the MacFarland scale, the suspension was then diluted in culture medium and, if the turbidity was lower, a higher amount of inoculum was added.

The MIC was determined in sterile 96 microplates for tissue culture (TPP) in a final volume of 200µL. In each plate orifices were added 192µL of Müeller-Hinton broth, 6µL of the rotenoid sample and 2µL of the bacteria or yeasts suspensions. The rotenoids were diluted in absolute ethanol in different concentrations at 35.0µg mL^{-1} to 1400.0µg mL^{-1}. In the blank control, the same microorganism suspension was inoculated in 198µL of Müeller-Hinton broth. In the negative control, no microorganism was inoculated in a 194µL of culture broth and 6µL of ethanol. The microplates were inoculated for 24h in 36°C. The MIC determination employing Müller-Hinton broth with 150mM (0.85%) de NaCl the same procedures were employed.

Clitoriacetal [1]. Ultraviolet (UV, MeOH): 200 and 295 nm. Infrared (IR, KBr): 2933 (C-H), 1641 (C=O) cm^{-1}. ^{1}H NMR (Nuclear Magnetic Resonance, $CDCl_3$, 300 MHz, δ): 6.70 (1H, *s*, H-1), 6.5 (1H, *s*, H-4), 5.74 (1H, *J*= 2.1, *d*, H-6), 4.57 (1H, *J*= 2.4, *d*, H-6a), 5.98 (1H, *J*= 2.4, *d*, H-8), 6.07 (1H, *J*= 2.1, *d*, H-10), 6.07 (1H, *s*, OH-11), 3.78 (3H, *s,* OCH_3), 3.75 (3H, *s,* OCH_3), and 3.74 ppm (3H, *s,* OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz, δ): 109.1, 108.7, 144.2, 151.7, 101.8, 148.0, 91.4, 74.5, 160.8, 94.6, 169.0, 95.7, 164.3, 101.0, 193.3, 69.6, 56.2, 55.7, and 55.7 ppm. Retention factor in TLC (R*f*): 0.38 (silica gel, CH_2Cl_2: MeOH 95:5 v/v).

6-Desoxyclitoriacetal [2]. UV (MeOH): 200 and 295 nm. IR (KBr): 2848 (C-H) and 1709 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 6.69 (1H, *s*, H-1), 6.49 (1H, *s*, H-4), 4.56 (1H, *J*= 2.1, *d*, H-6), 4.48 (1H, *J*= 2.4, *d*, H-6a), 5.97 (1H, *J*= 2.5, *d*, H-8), 6.05 (1H, *J*= 2.5, *d*, H-10), 11.51 (1H, *s*, OH-11), 3.82 (3H, *s,* OCH_3), 3.77 (3H, *s,* OCH_3), and 3.75 ppm (3H, *s,* OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz, δ): 109.2, 108.2, 144.0, 151.3, 101.1, 148.3, 63.6, 75.5, 161.6, 94.5, 169.0, 95.6, 164.3, 100.1, 195.0, 66.9, 56.3, 55.8, and 55.8 ppm. R*f*: 0.64 (silica gel, CH_2Cl_2: MeOH 95:5 v/v). Melting point (*mp*): 129-130 °C. Electron Impact Mass Spectrometry (EIMS, 70 eV): *m/z* 374 [M^+].

Stemonal [3]. UV (MeOH): 200, 273 and 321 nm. ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 8.43 (1H, *s*, H-1), 6.72 (1H, *s*, H-4), 6.48 (1H, *J*= 2.1, *d*, H-6), 6.44 (1H, *J*= 2.4, *d*, H-8), 6.4 (1H, *J*= 2.4, *d*, H-10), 12.83 (1H, *s*, OH-11),

6.07 (1H, *s*, OH-6), 3.97 (3H, *s,* OCH_3), 3.91 (3H, *s,* OCH_3), and 3.88 ppm (3H, *s,* OCH_3). R*f*: 0.54 (silica gel, CH_2Cl_2:MeOH 95:5 v/v). ***mp*:** 110-112 °C.

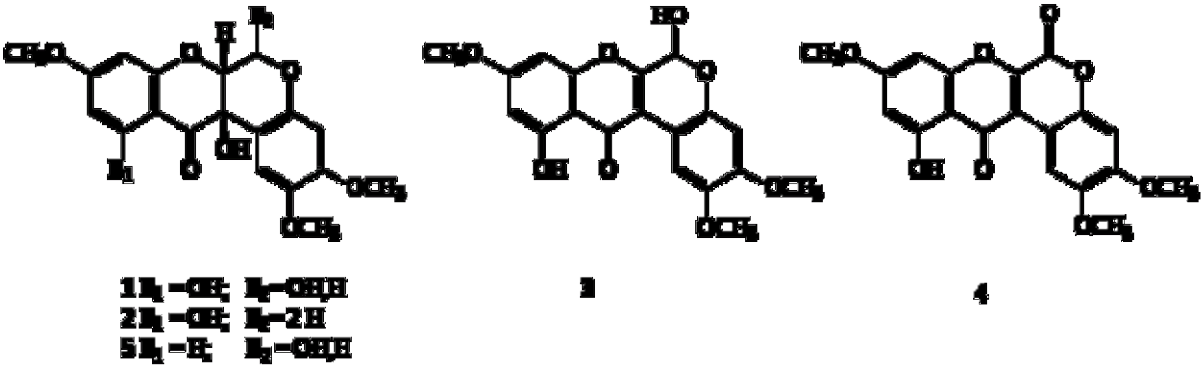
Stemonone [4]. UV (MeOH): 254, 281 and 334 nm. ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 8.9 (1H, *s*, H-1), 6.9 (1H, *s*, H-4), 6.4 (1H, *J*= 2.1, *d*, H-8), 6.6 (1H, *J*= 2.1, *d*, H-10), 12.4 (1H, *s*, OH-11), 4.0 (3H, *s,* OCH_3), 3.99 (3H, *s,* OCH_3), and 4.1 ppm (3H, *s,* OCH_3). R*f*: 0.86 (silica gel, CH_2Cl_2:MeOH 95:5 v/v).

11-Desoxyclitoriacetal [5]. UV (MeOH): 252 and 281 nm. ^{1}H NMR (MeOD, 600 MHz, δ): 7.79 (1H, *J*= 8.9, *d*, H-11), 6.7 (1H, *s,* H-1), 6.6 (1H, *s,* H-2), 6.46 (1H, *J*= 2.3, *d*, H-10), 6.14 (1H, *J*= 2.3, *d*, H-6), 5.88 (1H, *J*= 1, *d*, H-8), 4.68 (1H, *J*= 0.8, *d*, H-6a), 3.8 (3H, *s,* OCH_3), 3.7 (3H, *s,* OCH_3), and 3.6 ppm (3H, *s,* OCH_3).

Results and discussion

The rotenoids isolated from the seeds and roots of *C. fairchildiana* (**FIGURE 1**) were identified by NMR, IR, UV and MS spectral data analyzes and comparison with literature.

FIGURE 1: Strucutres of clitoriacetal[1], 6-desoxyclitoriacetal[2], stemonal[3], stemonone[4] e 11-desoxyclitoriacetal [5].



The presence of these rotenoids in different parts of *C. fairchildiana* was already described but this work is the first report about occurrence of compound 3 in seeds. Previously, stemonal was just isolated from the roots [11, 12].

There are few informations dealing with biological activities of rotenoids without the ring-E. The results observed for antimicrobial activities of these compounds were presently obtained. The isolates were tested against seven bacterias, two of them Gram-positive (*S. aureus* and *S. epidermidis*), one bacillus Gram-positive (*B. subtilis*), four microorganisms Gram-negative (*E. coli, E. aerogenes, P. mirabilis* and *P. aeruginosa*) and the yeasts *C. albicans, C. glabrata, C. kruzei* and *C. parapsilosis.* The MIC were calculated in culture medium with lower concentration of saline and culture medium with 150mM of NaCl. The results did not show difference. All the seven bacteria tested showed resistence at 1400µg mL^{-1} of rotenoid samples. The MIC of the yeasts ranged from 700µg mL^{-1} to 1050µg mL^{-1} (**TABLE 1**).

TABLE 1: Mininum inhibitory concentration (MIC) of rotenoids in Müeller-Hinton broth

Microorganisms	MIC (µg mL^{-1})				
	Compounds				
	1	**2**	**3**	**4**	**5**
Staphylococcus aureus CBAM324	R	R	R	R	R
Staphylococcus epidermidis ATCC 12228	R	R	R	R	R
Bacillus subtilis WT	R	R	R	R	R
Escherichia coli CBAM 002	R	R	R	R	R
Proteus mirabilis ATCC 15200	R	R	R	R	R
Enterobacter aerogenes ATCC 13048	R	R	R	R	R
Pseudomonas aeruginosa ATCC 25003	R	R	R	R	R
Candida albicans	700	R	R	R	1050
Candida glabrata	700	1050	R	R	700
Candida kruzei	700	1050	R	R	700
Candida parapsilosis	700	R	R	R	700

Fonte: R: Resistent.

Clitoriacetal [1] showed higher inhibition activity of the four yeasts when compared with compound 2. This result demonstrated a different correlation with anti-inflammatory and cytoxic activities observed for these two compounds [6, 7]. These findings corroborate biological activity is dependent of the *Cis* junction of Ring-B/C once stemonal and stemonone showed low activities. Besides, the number of hydroxyl groups also seems to interfere in the higher antimicrobial activities.

Conclusions

In this work rotenoids were isolated from seeds and roots and they showed antifungal activities, but no bacterial activities were detected at rotenoid concentrations lower than 1400µg mL^{-1}. This work relates for the first time these compounds were tested against bacteria and yeasts.

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