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Artigo

Chemical Constituents and Antimicrobial Activity of Branches and Leaves of *Cordia insignis* (Boraginaceae)

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Constituintes Químicos e Atividade Antimicrobiana de Ramos e Folhas de *Cordia insignis* (Boraginaceae)

Resumo: A investigação fitoquímica dos galhos e folhas da espécie *Cordia insignis* levou ao isolamento e identificação de ***β***-sitosterol (**1**), estigmasterol (**2**), campesterol (**3**), 3-O-*β*-D-glicopiranosil sitosterol (**4**), 3-O-*β*-D- glicopiranosil estigmasterol (**5**), escoparona (**6**), α-amirina (**7**), β-amirina (**8**), cafeoato de metila (**9**), alantoína (**10**), escopoletina (**11**), ácido cafeico (**12**) e rosmarinato de metila (**13**). A determinação estrutural foi feita através de dados de RMN (1D e 2D) e CG-EM e comparando com os dados da literatura. Os extratos hidroetanólicos dos galhos e folhas e suas respectivas frações foram submetidos à avaliação antibacteriana e antifúngica. Os extratos e frações apresentaram boa atividade antifúngica, porém não apresentaram atividade antibacteriana. Este estudo é o primeiro trabalho com a espécie *C. insignis*.

Palavras-chave: *Cordia insignis;* metabólitos secundários; atividade antifúngica.

Abstract

Phytochemical investigation of the branches and leaves of the species *Cordia insignis* led to the isolation and identification of ***β***-sitosterol (**1**), stigmasterol (**2**), campesterol (**3**), β-sitosterol 3-O-β-D-glucopyranoside (**4**), stigmasterol 3-O-β-D-glucopyranoside (**5**), scoparone (**6**), α-amyrin (**7**), β-amyrin (**8**), methyl caffeate (**9**), allantoin (**10**), scopoletin (**11**), caffeic acid (**12**), methyl rosmarinate (**13**). These substances had their structures determined by analysis of their 1D and 2D NMR and GC-MS spectra and by comparison with data reported in the literature. The hydroethanolic extracts of the branches and leaves and their respective fractions were submitted to antibacterial and antifungal evaluation. The extracts and fractions showed good antifungal activity but did not present antibacterial activity. This study is the first work with *C. insignis*.

Keywords: *Cordia insignis;* secundary metabolites; antifungal activity.

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Chemical Constituents and Antimicrobial Activity of Branches and Leaves of *Cordia insignis* (Boraginaceae)

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1. Introduction

The genus *Cordia* (Boraginaceae) which comprise of 320 species found in temperate and subtropical regions, but mainly in South America. They are distributed throughout Brazilian territory, with emphasis on the Atlantic and Cerrado forest biomes.[1] In several *Cordia* species the following classes of metabolites were identified: triterpenes, flavonoids, saponins, quinones, terpenes, alkaloids, coumarins, phenyl propanoids, etc.[1, 2, 3] The major pharmacological activities reported for extracts and isolated compounds include anti-inflammatory, antioxidant, larvicidal, hepatoprotective, analgesic, antimicrobial and antidiabetic.[4] The methanolic extract of branches and leaves of *C. salicifolia* presented cytotoxic activity against cancer cells.[5] Aqueous and hydroalcoholic extract of *C. americana* were found to be the most effective against *herpes simplex* virus type 1.[6] The methanol extract of the bark *C. platythyrsa* showed significant anti-inflammatory effect in carrageenan-induced rat paw edema.[7] Ethanol bark extract of *C. rothii* demonstrated a significant hypoglycemic effect

when estimating fasting and fed blood glucose levels using Dextrostix kit.[8] The *Cordia insignis* species, popularly known as "calção-de-velho", is restricted to Brazil and is distributed in the Northeast, Midwest and Southeast regions in Cerrado environments.[9] In folk medicine its leaves are used in the form of tea or bottled in the treatment of general pains and rheumatism.[10] The present work is the first report about the chemical constituents and antibacterial and antifungal activities *in vitro* of *C. insignis*.

2. Experimental

2.1. General Procedures

All compounds were identified by one and two-dimensional Nuclear Magnetic Resonance - 1D and 2D NMR and/or Gas Chromatography Mass Spectrometry experiments and in comparison, with the literature. NMR experiments were acquired in $CDCl_3$, methanol-d_4 or DMSO-d_6 at 293 K on Bruker AM-500 500 NMR spectrometer operating at 14.1 Tesla, observing ^1H and ^{13}C at 500.13 MHz and 150.76 MHz, respectively, equipped with a 5-mm quadrinuclear inverse detection probe with z-gradient. Chemical shifts (δ) were expressed in ppm and coupling constants (*J*) in Hertz. NMR spectra were referenced by TMS. GC-MS were performed on a Shimadzu CGMS 5050 chromatograph, employing DB5 (30 m/0,25 mm) column (JW). Silica gel 60 (70-230 mesh) was used for column chromatography (CC) with 0.063-0.200 mm particles (Merck), while silica gel 60 F254 was used for thin-layer chromatography (TLC).

2.2. Plant Material

Cordia insignis (CGEN code AF8CO57) was collected in Chapada dos Guimarães, Mato Gosso State, Brazil in March 2014 (S 15°21.964' W 055°57.372'). A voucher specimen (41454) is available at the UFMT Central Herbarium (Cuiabá, Mato Grosso State, Brazil).

2.3. Extraction and isolation

Dried and ground branches (2.12 kg) and leaves (1.26 kg) of *C. insignis* were extracted separately with EtOH/H_2O (80:20) at room temperature. After concentration under reduced pressure were obtained hydroethanolic extract of the branches HEB (185.21 g) and leaves HEL (166.72 g). HEB (140.0 g) was suspended in 250 mL of MeOH: H_2O (80:20) and extracted with *n*-hexane (FHB, 7.73 g), $CHCl_3$ (FCB, 8.38 g), EtOAc (FAB, 38.60 g) and the hydromethanolic residue (FHMB, 84.25). The HEL (156.0 g) was solubilized in 500 mL of MeOH and 500 mL of distilled water and allowed to stand in the refrigerator for 48 h. After the solution was filtered to remove the chlorophyll. The hydromethanolic solution was extracted with n-hexane (FHL, 0.25 g), $CHCl_3$ (FCL, 3.95 g), EtOAc (FAL, 14.23 g) and the hydromethanolic residue (FHML, 98.3 g). All fractions, except FHL (low mass), were submitted to chromatography on silica gel (70-230 mesh) using *n*-C_6H_{14}, $CHCl_3$ or CH_2Cl_2, EtOAc and MeOH (pure or in increasing gradient of solvent polarity) and Sephadex LH-20 using MeOH or $CHCl_3$ (pure or in mixture). FHB (5.5 g) to afford mixture **1**, **2** and **3** (7.2 mg) through elution with *n*-C_6H_{14}-EtOAc (9:1) and mixture **4** and **5** (10.2 mg) on elution in *n*-C_6H_{14}-EtOAc (2:8). FCB (6.45 g) to give **6** (3 mg) from the elution $CHCl_3$-EtOAc (7:3). FAB (30.2 g) was subjected to CC on silica gel eluted $CHCl_3$-EtOAc and EtOAc-MeOH to obtain 8 fractions. Fraction 1 (421.3 mg) from the elution $CHCl_3$-EtOAc (7:3) was purified on Sephadex LH-20 column eluted with $CHCl_3$ to provide mixture of **7** and **8** (5.3 mg). Fraction 6 (1.98 g) was separated by CC (SiO_2; $CHCl_3$- EtOAc and EtOAc-MeOH) to give **9** (1.4 mg) through elution with $CHCl_3$-EtOAc (3:7). Fraction 7 (18.14 g) was submitted to CC (SiO_2; EtOAc-MeOH) to give **10** (15.8 mg) on elution in EtOAc:MeOH (8:2). **FCL** (3.0 g) was subjected to CC (gradient CH_2Cl_2-EtOAc and EtOAc-MeOH) obtained 7 fractions. Fraction 3 (16.5 mg) was separated by preparative TLC (CH_2Cl_2-EtOAc, 9:1) to yield **6** (4.5 mg) and **11** (5 mg). **FAL** (12 g) was subjected to CC (SiO_2, gradient EtOAc-MeOH) to afford 9 fractions. Fraction 3 (123 mg) eluted on EtOAc was purified by Sephadex LH 20 CC with MeOH yielding **12** (7.1 mg) and mixture of **12** and **13** (10.2 mg). Fraction 6 to give **10** (15 mg) in EtOAc: MeOH (8:2).

2.4. Antimicrobial assay

For the bioassays of the antimicrobial activity standardized strains were used originating from the American Type Culture Collection (ATCC)

against six standard fungal strains: *Candida albicans* (ATCC 90028), *C. glabrata* (ATCC 9030), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019), *C. tropicalis* (ATCC 760) and *Cryptococcus neoformans* (ATCC 32045); five Gram-positive bacterial strains: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC) 19615, *Bacillus subitilis* (6051), and five Gram-negative bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiela pneumoniae* (ATCC 700603), *Salmonella typhimurium* (ATCC 14028) and *Shigella flexneri* (ATCC 12022). Amphotericin B and Chloramphenicol were used as the reference antimycotic and antibacterial controls, respectively. The antifungal and antibacterial activities of extracts and fractions of *C. insignis* were determined using microbroth dilution assays in 96-well microplates, in duplicate.[11,12] The antimicrobial activity was detected using a colorimetric method by adding 25 µL of the resazurin staining (0.01 %) aqueous solution in each well at the end of the incubation period.[13,14] The minimal inhibitory concentration (MIC) was defined as the lowest extract concentration able to inhibit the microbial growth, as indicated by resazurin staining.

3. Results and Discussion

3.1. Isolated compounds

Chemical investigation of leaves and branches of *C. insignis* resulted in the isolation and identification of 13 compounds (Figure 1), including: three steroids, β-sitosterol (**1**), stigmasterol (**2**), campesterol (**3**),[15] two saponins β-sitosterol 3-O-β-D-glucopyranoside (**4**), stigmasterol 3-O-β-D-glucopyranoside (**5**), [16,17] two coumarins, scoparone (**6**),[18] scopoletin (**11**),[19] two triterpenes α-amyrin (**7**), β-amyrin (**8**),[20] three phenylpropanoic derivates methyl caffeate (**9**),[21] caffeic acid (**12**),[22] methyl rosmarinate (**13**),[23] and a ureide allantoin (**10**)[24] were identified by 1D and 2D NMR and GC-MS spectra and by comparison with the reported data in the literature.

***β*-sitosterol (1):** ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.51-3.57 (1H, *m,* H-3), 5.36 (1H, *sl*, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.6 (C-6), 31.6 (C-7,8), 50.2 (C-9), 36.1 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.8 (C-14), 24.2 (C-15), 28.2 (C-16), 56.0 (C-17), 12.1 (C-18), 19.3 (C-19), 40.4 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.6 (C-25), 19.7 (C-26), 19.0 (C-27), 23.1 (C-28), 11.9 (C-29); GC-MS: *m/z* 414 [$C_{29}H_{50}O$].

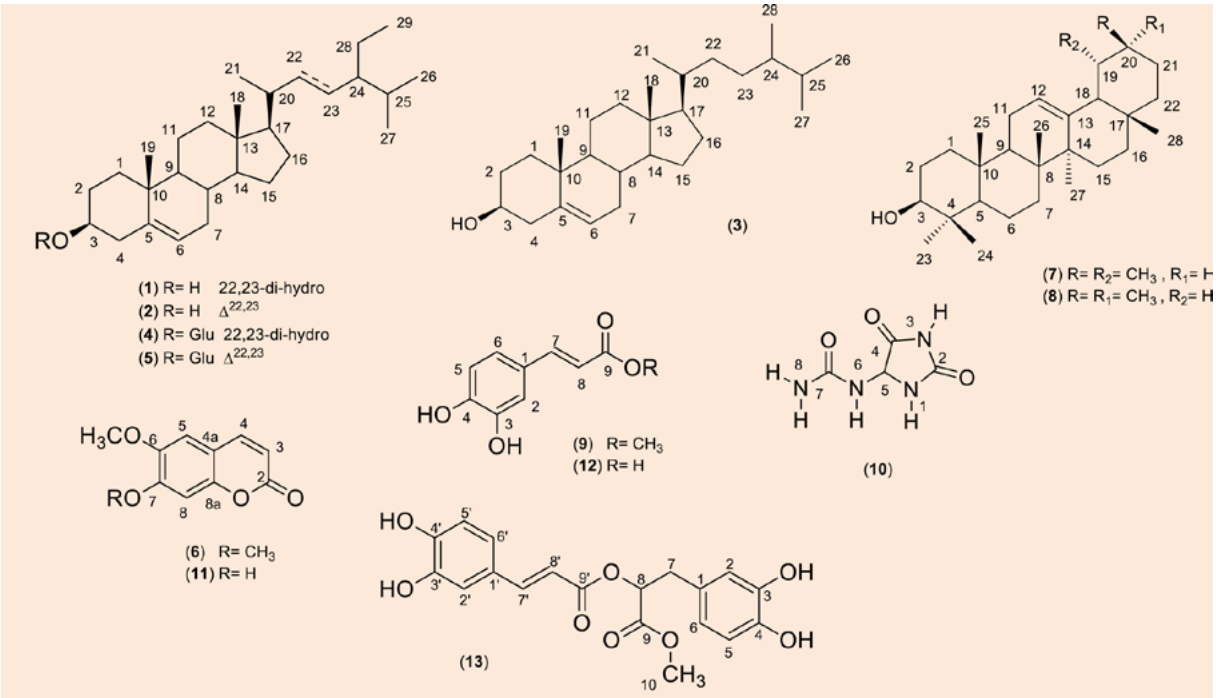

Figure 1. Structures of isolated compounds of *Cordia insignis*

Stigmasterol (2): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.51-3.57 (1H, *m,* H-3), 5.36 (1H, *sl*, H-6), 5.18 (1H, *dd*, J= 15.2, 8.6 Hz, H-22), 5.05 (1H, *dd*, J= 15.2, 8.6 Hz, H-23); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.6 (C-6), 31.6 (C-7,8), 50.2 (C-9), 36.1 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.8 (C-14), 24.2 (C-15), 28.2 (C-16), 56.0 (C-17), 12.1 (C-18), 19.3 (C-19), 40.4 (C-20), 20.1 (C-21), 138.2 (C-22), 129.3 (C-23), 50.1 (C-24), 31.9 (C-25), 22.6 (C-26), 19.7 (C-27), 25.3 (C-28), 11.8 (C-29). GC-MS: *m/z* 412 [$C_{29}H_{48}O$]

Campesterol (3): ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.51-3.57 (1H, *m,* H-3), 5.36 (1H, *sl*, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.6 (C-6), 31.6 (C-7,8), 50.2 (C-9), 36.1 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.8 (C-14), 24.2 (C-15), 28.2 (C-16), 56.0 (C-17), 12.1 (C-18), 19.3 (C-19), 40.4 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.6 (C-25), 19.7 (C-26), 19.0 (C-27), 23.1 (C-28). GC-MS: *m/z* 400 [$C_{28}H_{48}O$]

β-sitosterol 3-O-β-D-glucopyranoside (4): ^{1}H-NMR (400 MHz, C_5D_5N) δ: 3.82-3.91 (1H, *m*, H-3), 5.35 (1H, *sl*, H-6), 4.87 (1H, *dl*, J= 7.7 Hz, H-1'), 3.98-4.05 (2H, m, H-2', 3'), 4.32-4.38 (2H, *m*, H-4', 5'), 4.17 (2H, *dd*, J= 11.7, 5.4 Hz), H-6'; ^{13}C-NMR (125 MHz, C_5D_5N) δ: 37.2 (C-1), 30.0 (C-2), 78.2 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.7 (C-10), 21.0 (C-11), 39.1 (C-12), 42.1 (C-13), 56.6 (C-14), 24.2 (C-15), 28.3 (C-16), 56.0 (C-17), 11.7 (C-18), 19.0 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.2 (C-25), 19.7 (C-26), 19.2 (C-27), 23.1 (C-28), 11.9 (C-29), 102.3 (C-1'), 75.0 (C-2'), 77.9 (C-3'), 71.4 (C-4'), 78.3 (C-5'), 62.6 (C-6').

Stigmasterol 3-O-*β*-D-glucopyranoside (5): ^{1}H-NMR (400 MHz, C_5D_5N) δ: 3.82-3.91 (1H, *m*, H-3), 5.35 (1H, *sl*, H-6), 5.20 (1H, *dd*, J= 15.1, 8.7 Hz, H-22), 5.05 (1H, *dd*, J= 15.1, 8.7 Hz, H-23), 4.58-3.60 (6H, *m*, H-1',2',3',4',5',6'): ^{13}C-NMR (125 MHz, C_5D_5N) δ: 37.2 (C-1), 30.0 (C-2), 78.2 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.7 (C-10), 21.0 (C-11), 39.1 (C-12), 42.1 (C-13), 56.6 (C-14), 24.2 (C-15), 28.3 (C-16), 56.0 (C-17), 11.7 (C-18), 19.0 (C-19), 36.1 (C-20), 18.8 (C-21), 138.6 (C-22), 129.2 (C-23), 45.8 (C-24), 29.2 (C-25), 19.7 (C-26), 19.2 (C-27), 23.1 (C-28), 11.9 (C-29), 102.3 (C-1'), 75.0 (C-2'), 77.9 (C-3'), 71.4 (C-4'), 78.3 (C-5'), 62.6 (C-6').

Scoparone (6): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.32 (1H, *d*, J= 9.45 Hz, H-3), 7.65 (1H, *d*, J= 9.45 Hz, H-4), 6.83 (1H, *s*, H-5), 7.82 (1H, *s*, H-8), 3.95 (3H, *s*, -OCH_3), 3.98 (3H, *s*, -OCH_3); DEPT 135 (125 MHz, $CDCl_3$) δ: 161.4 (C-2), 113.5 (C-3), 143.3 (C-4), 111.4 (C-4a), 107.9 (C-5), 146.3 (C-6), 152.8 (C-7), 100.0 (C-8), 150.0 (C-8a), 56.4 (-OCH_3), 56.3 (-OCH_3) . GC-MS: *m/z* 206 [$C_{11}H_{10}O_4$].

α-amyrin (7): DEPT 135 (125 MHz, CD_3OD) δ: 38.4 (C-1), 28.3 (C-2), 78.3 (C-3), 38.9 (C-4), 55.3 (C-5), 18.1 (C-6), 32.4 (C-7), 39.1 (C-8), 48.4 (C-9), 36.7 (C-10), 23.1 (C-11), 125.7 (C-12), 138.5 (C-13), 41.4 (C-14), 26.5 (C-15), 26.4 (C-16), 33.5 (C-17), 55.5 (C-18), 41.3 (C-19, 20), 30.9 (C-21), 41.8 (C-22), 27.3 (C-23), 15.2 (C-24), 15.0 (C-25), 16.3 (C-26), 22.5 (C-27), 27.3 (C-28), 17.1 (C-29), 20.8 (C-30).

β-amyrin (8): DEPT 135 (125 MHz, CD_3OD) δ: 38.4 (C-1), 27.4 (C-2), 78.2 (C-3), 38.9 (C-4), 55.3 (C-5), 18.0 (C-6), 32.4 (C-7), 39.1 (C-8), 48.4 (C-9), 36.6 (C-10), 23.1 (C-11), 122.2 (C-12), 143.8 (C-13), 41.4 (C-14), 26.1 (C-15, 16), 32.6 (C-17), 46.2 (C-18), 45.8 (C-19), 31.6 (C-20), 34.5 (C-21), 38.7 (C-22), 27.3 (C-23), 14.9 (C-24), 14.4 (C-25), 16.3 (C-26), 24.9 (C-27), 27.3 (C-28), 34.2 (C-29), 22.5 (C-30).

Methyl caffeate (9): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.05 (1H, *d*, J= 2.0 Hz, H-2), 6.79 (1H, d, J= 8.2 Hz, H-5), 6.96 (1H, *dd*, J= 2.0, 8.2 Hz, H-6), 7.56 (1H, *d*, J= 15.9 Hz, H-7), 6.28 (1H, *d*, J= 15.9 Hz, H-8), 3.78 (3H, *s*); ^{13}C-NMR (125 MHz, CD_3OD) δ: 126.2 (C-1), 115.1 (C-2), 145.6 (C-3), 148.1 (C-4), 113.4 (C-5), 121.6 (C-6), 145.3 (C-7), 113.7 (C-8), 168.5 (C-9), 56.9 (-OCH_3).

Allantoin (10): ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.04 (1H, *sl*, H-1), 10.53 (1H, *s*, H-3), 5.25 (1H, *d*, J= 8.1 Hz, H-5), 6.89 (1H, *d*, J= 8.1 Hz, H-6), 5.77 (1H, *sl*, H-8); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 156.8 (C-2), 157.4 (C-4), 62.9 (C-5), 174.1 (C-7).

Scopoletin (11): ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.26 (1H, *d*, *J*= 9.4 Hz, H-3), 7.84 (1H, *d*, J= 9.4 Hz, H-4), 6.98 (1H, *s*, H-5), 8.50 (1H, *sl*, -OH), 6.99 (1H, *s*, H-8), 3.97 (3H, *s*, -OCH_3); ^{13}C-NMR (125 MHz, CD_3OD) δ: 168.8 (C-2), 112.1 (C-3), 143.9 (C-4), 111.5 (C-4a), 104.2 (C-5), 144.4 (C-6), 152.7 (C-7), 99.2 (C-8), 148.9 (C-8a), 55.4 (-OCH_3).

Caffeic acid (12): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.05 (1H, *d*, *J*= 2.0 Hz, H-2), 6.79 (1H, *d*, J= 8.2 Hz, H-5), 6.94 (1H, *dd*, J= 8.2, 2.0 Hz, H-6), 7.53 (1H, *d*, J= 15.9 Hz, H-7), 6.24 (1H, *d*, J= 15.9 Hz, H-8);

^{13}C-NMR (125 MHz, CD_3OD) δ: 126.5 (C-1), 113.6 (C-2), 145.1 (C-3), 145.3 (C-4), 115.0 (C-5), 121.3 (C-6), 147.9 (C-7), 116.3 (C-8), 170.0 (C-9).

Methyl rosmarinate (13): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.07 (1H, *d*, J= 2.1 Hz, H-2'), 6.79 (1H, *d*, J= 8.1 Hz, H-5'), 6.98 (1H, *dd*, J= 8.3, 2.0 Hz, H-6'), 7.57 (1H, *d*, J= 15.9 Hz, H-7'), 6.28 (1H, *d*, J= 15.9 Hz, H-8'), 5.21 (2H, *dd*, J= 7.6, 5.5 Hz, H-8), 3.03 (1H, *dd*, J= 14.4, 7.7 Hz, H-7a), 3.08 (1H, *dd*, J= 14.8, 5.8 Hz, H-7b), 6.59 (1H, *dd*, J= 8.1, 2.1 Hz, H-6), 6.72 (1H, *d*, J= 8.1 Hz, H-5), 6.73 (d, J= 2.1 Hz, H-2) 3.72 (3H, *s*). ^{13}C-NMR (125 MHz, CD_3OD) δ: 126.1 (C-1'), 113.8 (C-2'), 146.5 (C-3'), 145.4 (C-4'), 115.1 (C-5'), 121.8 (C-6'), 148.4 (C-7'), 112.7 (C-8'), 166.9 (C-9'), 170.7 (C-9), 73.2 (C-8), 36.5 (C-7), 120.4 (C-6), 115.0 (C-5), 144.8 (C-4), 143.9 (C-3), 116.1 (C-2), 127.3 (C-1), 51.2 (-OCH_3).

3.2. Antimicrobial bioassay

The crude hydroethanolic extracts were evaluated against the fungal and bacterial strains described in this work. It can be observed that no extract or fraction showed promising activity against the bacterial strains. For the fungal strains (Table 1), the extract of leaves (**HEL**) and fractions *n*-hexane (**FHL)**, $CHCl_3$ (**FCL**) and ethyl acetate (**FAL**) were active against *C. albicans*, *C. glabrata* and *C. krusei* strains, with MIC ranging from 15.62 to 250 µg /mL. The **FHL**, **FCL** and **FAL** fractions also presented significant results against the *C. neoformans* strain, with MIC ranging from 125 to 500 µg /mL. The extract of branches (**HEB**) and $CHCl_3$ (**FCB**) fraction presented promising results against the *C. albicans* strain, with MIC of 500 and 125 µg/mL, respectively. The ethyl acetate (**FCB)** fraction was also active against *C. glabrata* strains with MIC 500 µg/mL. These promising results may be related to the presence of substances that present antifungal activities.

4. Conclusion

The phytochemical investigation of *Cordia insignis* extracts led to the isolation and identification of thirteen compounds. The present work is the first report about the chemical constituents and antibacterial and antifungal activities *in vitro* of *C. insignis*. These substances are in accordance with the chemistry of the genus *Cordia* and these results suggest that *C. insignis* provides initial evidence for a new and alternative source of substances of medicinal interest with respect to antifungal activity.

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Table 1. Antifungal activity of crude extract and fractions from C. insignis (MIC µg/mL)

Extracts/ fractions	*C. albicans*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*
HEB	500	-	-	-	-	-
FHG	-	-	-	-	-	-
FCB	125	500	-	-	-	-
FAB	-	-	-	-	-	-
FHMB	-	-	-	-	-	-
HEL	250	500	250	-	-	-
FHL	15.6	250	125	-	-	-
FCL	125	500	500	-	-	500
FAL	62.5	125	250	-	-	250
FHML	-	-	-	-	-	-
AMP B	0.50	0.25	0.50	0.25	0.25	0.25

(-) No activity; AMP B: amphotericin B

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