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Original Article

Antifungal activity of extracts and phenolic compounds from *Deguelia duckeana*

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ABSTRACT

Candida spp. is associated with almost 80% of all nosocomial fungal infections and is considered a major cause of blood stream infections. In humans, Cryptococcosis is a disease of the lungs caused by the fungi *Cryptococcus gattii* and *Cryptococcus neoformans*. It can be potentially fatal, especially in immune-compromised patients. In a search for antifungal drugs, *Deguelia duckeana* extracts were assayed against these two fungi and also against *Candida albicans*, which causes candidiasis. Hexane branches and CH_2Cl_2 root extracts as well as the substances 4-hydroxylonchocarpine, 3,5,4′-trimethoxy-4-prenylstilbene and 3′,4′-methylenedioxy-7-methoxyflavone were assayed to determine the minimal inhibitory concentration. Phytochemical study of CH_2Cl_2 root and hexane branch extracts from *D. duckeana* A.M.G. Azevedo, Fabaceae, resulted in the isolation and characterization of nine phenolic compounds: 4-hydroxyderricine, 4-hydroxylonchocarpine, 3′,4′,7-trimethoxy-flavonol, 5,4′-dihydroxy-isolonchocarpine, 4-hydroxyderricidine, derricidine, 3,5,4′-trimethoxy-stilbene, 3′,4′,7-trimethoxyflavone and yangambin. The only active extract was a CH_2Cl_2 root showing minimal inhibitory concentration 800 μg/ml against *C. gattii*, and the investigation of compounds obtained from this extract showed that 4-hydroxylonchocarpine was active against all three fungi (*C. neoformans*, *C. gattii* and *C. albicans*). These results suggest that *D. duckeana* extracts have potential therapeutic value for the treatment of pathogenic fungi.

Introduction

Candida spp. is associated with almost 80% of all nosocomial fungal infections and is considered the major cause of blood stream infections and its infections involve a broad spectrum of superficial and invasive diseases. The result is a great medical challenge, due to both the difficulties of diagnosis and in finding effective countermeasures to the infections caused by these fungi (Colombo and Guimarães, 2003).

The Fabaceae is a large botanical family and a producer of phenolic compounds such as flavonoids and isoflavonoids used as chemotaxonomic markers (Hegnauer and Gpayer-Barkmeijer, 1993; Veitch, 2013). Species from Papilionoideae subfamily are known to produce substances with pharmacological properties, including flavonoids from *Tephrosia apollinea* with antifungal activity (Ammar et al., 2013), flavonoids from *Dalbergia odorifera* (Lee et al., 2013), and isoflavonoids from *Abrus mollis*, both with anti-inflammatory activity (Chen et al., 2014a).

Deguelia is one of some 750 genera in the Fabaceae. Studies of the members of this genus (sometimes under synonymies) report stilbene and flavanones from *Derris rariflora* (=*Deguelia rariflora*) (Braz Filho et al., 1975a); rotenone and tephrosin from *Derris urucu* (=*Deguelia rufescens* var. *urucu*) (Braz Filho et al., 1975a); isoflavan from *Derris amazonica* (=*Lonchocarpus negrensis*) (Braz Filho et al., 1975a); stilbene, lonchocarpine and 4-hydroxy-lonchocarpin from *Derris floribunda* (Braz Filho et al., 1975b); stilbene from *Deguelia spruceana* (Garcia et al., 1986); isoflavonoids from *Derris glabrescens* (=*Lonchocarpus densiflorus*) (Monache et al., 1977); prenylated isoflavonoids (Magalhães et al., 2001) and flavanone from *Deguelia hatschbachii* (Magalhães et al., 2003); prenylated flavonoids from *Deguelia longeracemosa* (Magalhães et al., 2006); dihydroflavonols from *D. urucu* (Lôbo et al., 2009), stilbenes from *D. rufescens* var. *urucu* (Lôbo et al., 2010); isoflavonoids and chromones (Lawson

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et al., 2008), chalcones and rotenoids from *Lonchocarpus nicou* (Lawson et al., 2010); flavonoids from *Deguelia utilis* (Oliveira et al., 2012) and stilbenes from *D. rufescens* (= *Derris urucu*, *Lonchocarpus urucu*) (Pereira et al., 2012). The main characteristic of this genus and its close relatives is the presence of isoprenyl groups but, as a recent review describes (Marques et al., 2015), it also possesses dimethylchromone and related compounds.

Deguelia duckeana A.M.G. Azevedo, Fabaceae, a species endemic to Brazil, is known as "cipó-cururu" or "timbó" and used by indigenous people to kill fish. It is known only from the Brazilian states of Amazonas, Pará and Rondônia (Camargo and Tozzi, 2017). As far as we know from the available literature, there are only three studies published concerning biological activity and/or chemical isolation of *D. duckeana*. One showing extract antimycobacterial activity (Carrion et al., 2013), another the presence of stilbene and chalcones, *Artemia salina* toxicity and moderate activity against *Staphylococcus aureus* (Lima et al., 2013) and a third describing the isolation of flavones, flavanones, chalcones and stilbene and their effect on cellular viability, AMPK, eEF2, eIF2 and eIF4E (Cursino et al., 2016).

Accordingly, the current study was carried out to enhance knowledge of the chemical and biological potential of *D. duckeana*. First, the antifungal activity of root and branch extracts was evaluated against *Cryptococcus gattii*, *C. neoformans* and *Candida albicans*. Thereafter, phytochemical fractionation of these extracts was performed to obtain pure compounds. As 4-hydroxylonchocarpine is described in the literature with activity against fungi (Mbaveng et al., 2008; Dzoyem et al., 2013; Kuete et al., 2013), this chalcone, together with 3′,4′-methylenedioxy-7-methoxyflavone and 3,5,4′-trimethoxy-4-prenylstilbene, all three previously isolated (Cursino et al., 2016), were assayed against *C. albicans* which causes candidiasis, a widespread disease (Chakravarthi and Haleagrahara, 2011), and against *C. gattii* and *C. neoformans* which caused Cryptococcosis, a serious disease—notably in immuno-compromised patients. *C. gattii* also causes meningoencephalitis and other central nervous system and pulmonary-linked diseases, which can often be fatal (Chen et al., 2014b).

Materials and methods

General experimental procedure

Spectral data were obtained from Varian Inova (^{1}H NMR 500 MHz) and Bruker DRX (^{1}H NMR 400 MHz). Samples were analyzed using $CDCl_3$ as solvent and internal standard. Compounds **8** and **9** were also analyzed by LC-MS MicroTOF-QII (Brucker Daltonics), ESI, positive mode and Prominence UFLC (Shimadzu) (DAD) SPDM-20A. The SiO_2 60 chromatography column (230–400 mesh) used was made by Merck, Germany, and the Sephadex LH-20 by Sigma. The solvents MeOH, hexane, EtOAc and CH_2Cl_2 were from Vetec. TLC of SiO_2 (UV254, 0.20 mm, Macherey, Nagel, USA).

Reference fungal strains

Candida albicans (ATCC 36232), *Cryptococcus neoformans* (WM 148, genotype VNI) and *Cryptococcus gattii* (WM 178, genotype VGII) were used as reference material. These strains were kindly supplied by the fungus collection held by Fiocruz-Rio de Janeiro, Brazil, and are now preserved in the microbial collection of the National Institute of Amazon Research (INPA), Manaus, Brazil. The cultures were preserved in mineral oil, and subcultures maintained on Sabouraud medium to ensure purity and viability until testing was performed.

Plant material

Roots and branches of *Deguelia duckeana* A.M.G. Azevedo, Fabaceae, were collected on Praia Dourada (Manaus, Amazonas, Brazil) in September 2005. In order to obtain more plant material to perform the chemical fractionation, a new collection was made in August 2009. Vouchers of both plant materials were deposited in the herbarium of Instituto Federal de Educação do Amazonas (EAFM), as accession numbers 10606 and 10613, respectively.

Plant extraction and substances isolation

Roots were dried and extracted with CH_2Cl_2 as solvent, using an ultrasound bath for 20 min (Unique, São Paulo, Brazil), filtered and the procedure repeated twice. Plant material was then dried and then extracted with methanol (MeOH), and finally with H_2O, with all extractions using the same procedure.

Dichloromethane root extract (8 g) was fractionated in a SiO_2 chromatography column with solvents hexane, CH_2Cl_2, EtOAc and MeOH as gradient. Combined fractions 20–30 obtained as medium polarity (EtOAc) were re-fractionated with CH_2Cl_2, CH_2Cl_2/EtOAc and EtOAc/MeOH. TLC preparative analysis of fraction **5** was eluted with CH_2Cl_2/EtOAc 95:5 and showed compounds in mixture (4.1 mg) as **1** and **2**.

Combined fractions 13–15 were purified by open column chromatography using a Sephadex LH-20 with MeOH as elution system yielding compound **3** and a mixture (128 mg) with compounds **2** (∼34%), **4** (∼26%) and **5** (∼40%). NMR spectral data allowed the correct identification of compounds without isolating them. Relative percentages were calculated in mixture by using ^{1}H NMR integration signals.

Combined fractions 4–5 (2.8 g) obtained from the first fractionation of CH_2Cl_2 root extract were separated with SiO_2 with the solvents hexane, EtOAc and MeOH yielding 50 fractions. Among them, fraction 39 was analyzed by LC-MS on a C-18 analytic column, using a gradient system with ACN/H_2O (0.1% acetic acid) 20% (0–11 min), 100% (11–12 min), 20% (12–15 min) and flow of 0.4 ml/min. The chromatogram showed two peaks at 5.8 and 6 min, corresponding to m/z 313.107121 $[M + H]^+$ ion (molecular formula $C_{18}H_{16}O_5$) for compound **8**, and m/z 469.182066 $[M + Na]^+$ (molecular formula $C_{24}H_{30}O_8$) for compound **9**.

In order to identify bioactive flavonoids, the hexanic branch extract (2 g) was fractionated with open column chromatography using SiO_2 with solvents hexane, EtOAc and MeOH as gradient. The combined fractions containing flavonoids was obtained using hexane/EtOAc 9:1 until 1:9 as the elution system, yielding compounds **6** (5 mg) and **7** (3.6 mg).

Fractionation of all samples were monitored by ^{1}H NMR, UV (254 and 365 nm), with reagents $FeCl_3$, $AlCl_3$ and $Ce(SO_4)_2$.

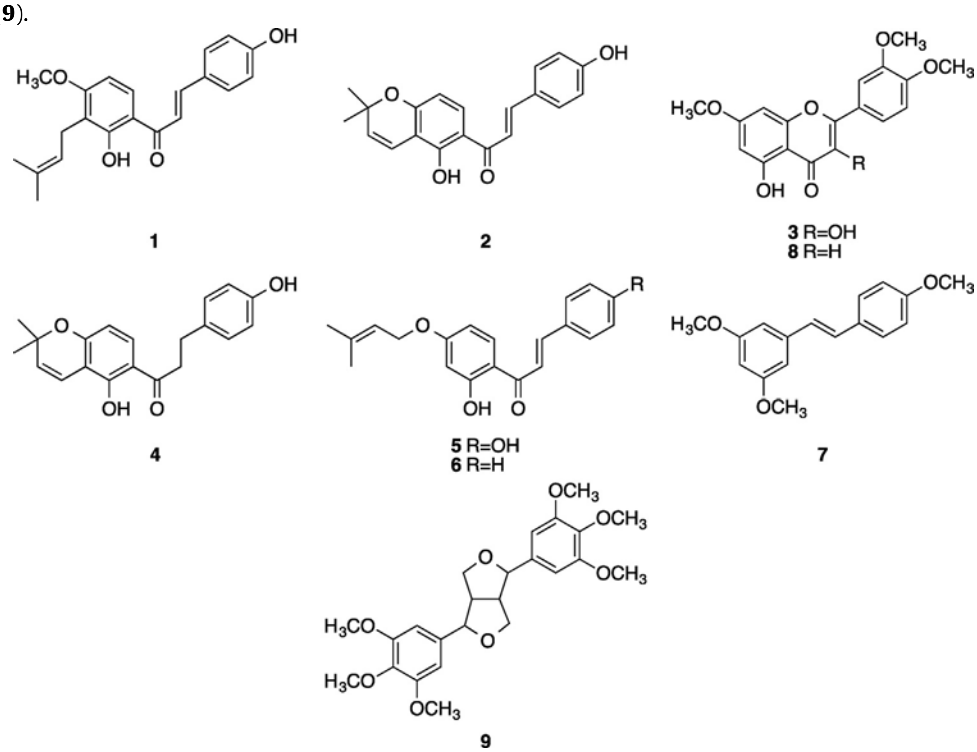
Antifungal activity

Previously isolated compounds (Cursino et al., 2016) were tested in the current study. Only three compounds (4-hydroxylonchocarpine, 3,5,4′-trimethoxy-4-prenylstilbene and 3′,4′-methylenedioxy-7-methoxyflavone) were selected because the first has cytotoxic activity reported in the literature and only they showed enough amount. In addition to these three, hexane branch and CH_2Cl_2 root extracts were also assayed to determine the minimal inhibitory concentration (MIC) as set by the Clinical and Laboratory Standards Institute 2008 (CLSI, 2008). Assays were performed in 96-well plates, each containing 100 μl of each previously diluted substance or extract, plus 100 μl of RPMI 1640 broth medium with substance or extract and 100 μl of diluted microorganism containing 2.5×10^3 CFU/ml. We evaluated concentrations from 800 to 6.25 μg/ml for plant extracts, and concentrations from 320 to 0.625 μg/ml for isolated substances. *C. albicans* was incubated at 35 °C for 24 h, and *Cryptococcus gattii* and *C. neoformans* at 35 °C for 72 h. Cultivated fungal strains and RPMI 1640 medium were used as negative controls, and amphotericin B (64 μg/ml) as a positive control. Dimethyl sulfoxide was used for compound dilution with final concentration in the bioassay below 1%. MIC values were determined visually after 24 h incubation, as the lowest concentration of drug that resulted in both ≥50% inhibition and 100% inhibition of growth relative to the growth of the control, as previously described by the Clinical and Laboratory Standards Institute 2008 (CLSI, 2008).

Results

Compound identifications

Phytochemical study of CH_2Cl_2 root and hexane branch extracts from *D. duckeana* resulted in isolation and characterization of nine phenolic compounds: 4-hydroxyderricine (**1**), 4-hydroxylonchocarpine (**2**), 3′,4′,7-trimethoxy-flavonol (**3**), 5,4′-dihydroxy-isolonchocarpine (**4**), 4-hydroxyderricidine (**5**), derricidine (**6**), 3,5,4′-trimethoxy-stilbene (**7**), 3′,4′,7-trimethoxyflavone (**8**) and yangambin (**9**).



Chalcone 4-hydroxyderricine (**1**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.68 (3H, *s*, Me), 1.80 (3H, *s*, Me), 3.38 (2H, *d*, *J* = 7.2 Hz, H-1″), 3.91 (3H, *s*, OMe), 5.23 (1H, *m*, H-2″), 6.49 (1H, *d*, *J* = 9.2 Hz, H-5′), 6.88 (1H, *d*, *J* = 8.8 Hz, H-3 and H-5), 7.47 (1H, *d*, *J* = 15.2 Hz, H-α), 7.56 (1H, *d*, *J* = 8.8 Hz, H-2 and H-6), 7.79 (1H, *d*, *J* = 9.2 Hz, H-6′), 7.83 (1H, *d*, *J* = 15.2 Hz, H-β), 13.45 (1H, *s*, 2′-OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 17.8 (C-5″), 21.6 (C-1″), 25.8 (C-4″), 102.0 (C-5′), 114.7 (C-1′), 115.9 (C-3, C-5), 117.4 (C-3′), 118.3 (C-α), 122.0 (C-2″), 127.7 (C-1), 129.1 (C-6′), 131.9 (C-3″), 130.6 (C-2 and C-6), 144.1 (C-β), 158.0 (C-4), 163.0 (C-2′), 163.2 (C-4′), 192.4 (C=O).

Chalcone 4-hydroxylonchocarpine (**2**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.45 (6H, *s*), 5.57 (1H, *d*, *J* = 10 Hz), 6.38 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.74 (1H, *d*, *J* = 10 Hz), 6.89 (2H, *d*, *J* = 8.5 Hz), 7.56 (2H, *d*, *J* = 8.5 Hz), 7.54 (1H, *d*, *J* = 15.5 Hz, H-α), 7.72 (1H, *d*, *J* = 8.5 Hz, H-6′), 7.85 (1H, *d*, *J* = 15.5 Hz, H-β), 13.75 (1H, *s*).

Flavonol 3′,4′,7-trimethoxy-flavonol (**3**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.96 (3H, *s*, OMe), 3.97 (3H, *s*, OMe), 3.99 (3H, *s*, OMe), 6.97 (1H, *d*, *J* = 8.0 Hz), 7.00 (1H, *d*, *J* = 2.0 Hz), 7.02 (1H, *dd*, *J* = 9.0 and 2.0 Hz), 7.39 (1H, *d*, *J* = 2.5), 7.59 (1H, *dd*, *J* = 8.5 and 2.0 Hz), 8.15 (1H, *d*, *J* = 8.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 55.9 (OCH_3), 56.0 (OCH_3), 56.3 (OCH_3), 100.1 (C-8), 110.7 (C-5′), 112.2 (C-2′), 115.0 (C-6), 116.2 (C-10), 123.1 (C-6′), 124.0 (C-1′), 127.8 (C-5), 148.6 (C-3′), 151.3 (C-4′), 152.4 (C-9), 160.0 (C-2), 164.5 (C-7), 172.5 (C=O).

5,4′-Dihydroxy-isolonchocarpine (**4**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 12.27 (1H, *s*), 7.30 (2H, *dd*, *J* = 8.5 and 2.5 Hz; H-2′ and H-6′), 6.88 (2H, *dd*, *J* = 8.5 and 2.5 Hz; H-3′ and H-5′), 6.61 (1H, *d*, *J* = 10.0, H-4″), 5.49 (1H, *d*, *J* = 10.0, H-3″) and 1.43 (6H, *s*), 5.95 (1H, *s*), 5.32 (1H, *dd*, *J* = 13 and 3.0 Hz, H-2), 3.06 (1H, *dd*, *J* = 17.0 and 13.0 Hz, H-3), 2.77 (1H, *dd*, *J* = 17.0 and 3.0 Hz, H-3).

Chalcone 4-hydroxyderricidine (**5**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.88 (2H, *d*, *J* = 8.5, H-3 and H-5), 7.54 (2H, *d*, *J* = 8.5, H-2 and H-6), 7.42 (1H, *d*, *J* = 15.5, H-α), 7.83 (1H, *d*, *J* = 15.5, H-β), 7.81 (1H, *d*, *J* = 8.5, H-6′), 6.47 (1H, *d*, *J* = 2.5, H-3′), 6.49 (1H, *dd*, *J* = 8.5 and 2.5, H-5′), 4.56 (2H, *d*, *J* = 7.0, H-1″), 5.49 (1H, *m*, H-2″), 1.80 (3H, *s*, CH_3), 1.75 (3H, *s*, CH_3), 13.56 (1H, *s*).

Chalcone derricidine (**6**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.44 (1H, *s*), 7.89 (1H, *d*, *J* = 16.0 Hz), 7.59 (1H, *d*, *J* = 16.0 Hz), 7.65 (2H, *m*, H-2 and H-6), 7.44 (3H, *m*, H-3, H-4 and H-6), 1.81 (3H, *s*), 1.76 (3H, *s*), 4.57 (2H, *d*, *J* = 6.8 Hz), 5.49 (1H, *m*), 6.50 (1H, *dd*, *J* = 8.4 and 2.4 Hz), 6.48 (1H, *d*, *J* = 2.4 Hz), 7.83 (1H, *d*, *J* = 8.4 Hz).

Stilbene 3,5,4′-trimethoxy-stilbene (**7**): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.04 (1H, *d*, *J* = 16.4 Hz, H-8), 6.91 (1H, *d*, *J* = 16.4 Hz, H-7), 3.83 (3H, *s*), 7.45 (2H, *dd*, *J* = 8.4 and 2.0 Hz, H-2′ and H-6′), 6.90 (2H, *d*, *J* = 8.4 and 2.0 Hz, H-3′ and H-5′), 3.832 (6H, *s*), 6.65 (2H, *d*, *J* = 2.0 Hz, H-2 and H-6), 6.37 (1H, *t*, *J* = 2.0 Hz, H-4).

Flavone 3′,4′,7-trimethoxyflavone (**8**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.92 (3H, *s*, 7-OCH_3), 3.95 (3H, *s*, 4′-OCH_3), 3.97 (3H, *s*, 3′-OCH_3), 6.98 (1H, *d*, *J* = 2.0, H-8), 6.98 (2H, *dd*, *J* = 8.5 and 2.0, H-6), 8.11 (1H, *d*, *J* = 8.5, H-5), 6.69 (1H, *s*, H-3), 7.53 (1H, *dd*, *J* = 8.5 and 2.0, H-6′), 6.96 (1H, *d*, *J* = 8.5, H-5′), 7.35 (1H, *d*, *J* = 2.0, H-2′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.8 (7-OCH_3), 56.0 (4′-OCH_3), 56.1 (3′-OCH_3), 117.6 (C-10), 157.8 (C-9), 111.0 (C-8), 164.0 (C-7), 114.2 (C-6), 126.9 (C-5), 177.8 (C-4), 106.3 (C-3), 163.0 (C-2), 119.8 (C-6′), 100.3 (C-5′), 151.8 (C-4′), 149.2 (C-3′), 108.7 (C-2′), 124.2 (C-1′).

Table 1
MIC (μg/ml) of extracts and isolated compounds from *Deguelia duckeana* against *Candida albicans*, *Cryptococcus gattii* and *Cryptococcus neoformans*.

	C. albicans (ATCC 36232)	*C. gattii* (WM 17)	*C. neoformans* (WM 148)
CH_2Cl_2 root extract	>800	800	>800
Hexanic branch extract	>800	>800	>800
4-Hydroxylonchocarpine	320	80	20
3′,4′-Methylenedioxy-7-methoxyflavone	>320	>320	>320
3,5,4′-Trimethoxy-4-prenylstilbene	>320	>320	>320
Amphotericin B	0.25	0.125	0.06

Lignan yangambin (**9**): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.09 (2H, *m*, 8, 8′), 3.82 (6H, *s*, OCH_3), 3.86 (12H, *s*, OCH_3), 3.92 (2H, *dd*, *J* = 9.0 and 6.9 Hz, H-9β, H-9′β), 4.31 (2H, *dd*, *J* = 9.0 and 6.9 Hz, H-9α, H-9′α), 4.75 (2H, *d*, *J* = 4.0, H-7, 7′), 6.57 (4H, *s*, H-2, H-6, H-2′, H-6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.3 (C-8, C-8′), 55.8 (OCH_3), 60.8 (OCH_3), 71.9 (C-9, C-9′), 85.9 (C-7, C-7′), 102.7 (C-2, C-6, C-2′, C-6′), 136.6 (C-1, C-1′), 137.4 (C-4, C-4′), 153.3 (C-3, C-3′, C-5, C-5′).

Although all these compounds are known, it is important to emphasize that *D. duckeana* has been reported as a species with important biological activities, but is so far very under-researched. The current study of *D. duckeana* found several phenolic compounds which corroborate known Fabaceae chemotaxonomy.

Antifungal activity

In terms of the MIC, as set by the Clinical and Laboratory Standards Institute 2008 (CLSI, 2008), the antifungal activity of 4-hydroxylonchocarpine showed significant results for members of the genera *Candida* and *Microsporum*, but the activity against *C. gattii*, described in the current study (Table 1), is being done so for first time, as far as we know.

Discussion

The identification of nine phenolic compounds from *D. duckeana* by the current study contributes to chemosystematic knowledge of genus *Deguelia*, which shows mostly flavonoid and related compounds. The compounds 4-hydroxylonchocarpine and derricidine were previously isolated from *D. duckeana* branches (Braz Filho et al., 1975b; Oliveira et al., 2012; Lima et al., 2013; Cursino et al., 2016; Ahmed et al., 2002), while 4-hydroxyderricine is described for the first time from the genus *Deguelia*.

Compound **1** (4-hydroxyderricine) was a yellow solid in mixture with compound **2** (4-hydroxylonchocarpine). Integration analysis of the signals from spectral data allowed the identification of compound **2** corresponding to 47.7% of the mixture. Two singlets at δ_H 13.45 and δ_H 13.75 indicate the presence of two flavonoids with chelated hydroxyl groups. Compound **1** was characterized as a chalcone due to two doublets at δ_H 7.47 (1H, *J* = 15.2 Hz) and δ_H 7.83 (1H, *J* = 15.2 Hz) related to the trans-olefinic hydrogens α and β, one singlet in δ_H 13.45 referable to a chelated hydroxyl group (C2′-OH), two doublets at δ_H 7.56 (2H, *J* = 8.8 Hz) and 6.88 (2H, *J* = 8.8 Hz) corresponding to H-2/H-6 and H-3/H-5, respectively. Two doublets at δ_H 7.79 (1H, *J* = 9.2 Hz, H-6′) and δ_H 6.49 (1H, *J* = 9.2 Hz, H-5′) indicated the presence of *ortho* coupling and one singlet at δ_H 3.91 (3H) characterized a methoxyl linked to an aromatic group. At δ_H 5.23 (1H, *m*), δ_H 3.38 (2H, *d*, *J* = 7.2 Hz), 1.80 (3H, *s*) and 1.68 (3H, *s*), a prenyl group was observed. The chemical shifts of carbon 4 (δ_C 158.0), 3 and 5 (δ_C 115.9) indicated that there was only a hydroxyl group linked to carbon 4. The structural proposal was confirmed by comparison with the literature (Shin et al., 2011).

Compound **2**, identified as chalcone 4-dihydroxylonchocarpine, is common in Fabaceae and Moraceae families. Chalcones are known to possess antimalarial (Ramírez et al., 2010), antibacterial, antifungal (Dzoyem et al., 2013) and anticancer (Ngameni et al., 2006) biological activity.

^{1}H NMR spectrum of compound **3** (3′,4′,7-trimethoxy-flavonol) showed *ortho* and *meta* hydrogens coupled with double doublets at δ_H 7.03 (*J* = 8.9 and 2.4 Hz) and 6.91 (*J* = 2.4 Hz) and a doublet at δ_H 8.20 (1H, *J* = 8.9 Hz) characterizing H-6, H-5 and H-8 of A-ring belonging to flavonoid nuclei, respectively.

In the ^{13}C NMR spectrum, 15 carbon sp^2 were observed, compatible with units of the C6-C3-C6 typical of flavonoids. The signal at δ_C 172.5 (C-4) is compatible with a flavonoid carbonyl group. The signals δ_C 112.2, 123.1 and 110.7 correlated on an HSQC contour map with δ_H 7.45 (*d*, *J* = 2.1 Hz), 7.57 (*dd*, *J* = 8.4 and 2.1 Hz) and 7.01 (*d*, *J* = 8.4 Hz), respectively, and indicated a B ring at the C-3 and C-4 positions. Verified signals δ_C 55.9, 56.0 and 56.3 on ^{13}C NMR correlated with singlets at δ_H 3.93, 3.97 and 3.98 on ^{1}H NMR and indicated the presence of three methoxyl aromatic groups which were assigned to C-7, C-3 and C-4 carbons. Localization of methoxyl substituent groups was confirmed by an HMBC contour map.

Compound **4** (5,4′-dihydroxy-isolonchocarpin) was recognized as a flavanone through signals of C-ring hydrogens [δ_H 5.32 (1H, *dd*, *J* = 13.0 and 3.0 Hz, H-2), 3.06 (1H, *dd*, *J* = 17.0 and 13.0 Hz, H-3), 2.77 (1H, *dd*, *J* = 17.0 and 3.0 Hz, H-3)]. One singlet at δ_C 12.27 characterized a chelated hydroxyl group that could be assigned to a flavanone C-5. Signals from a *para*-substituted B-ring [δ_H 7.30 (2H, *dd*, *J* = 8.5 and 2.5 Hz, H-2′ and H-6′) and 6.88 (2H, *dd*, *J* = 8.5 and 2.5 Hz, H-3′ and H-5′)], signals of gem-dimethyl-chromone [δ_H 6.61 (1H, *d*, *J* = 10.0, H-4″), 5.49 (1H, *d*, *J* = 10.0, H-3″) and 1.43 (6H, *s*)], and one singlet at H-8 [δ_H 5.95 (1H; *s*)] characterized the flavanone 5,4′-dihydroxy-isolonchocarpin.

Compound **5** (4-hydroxyderricidine) showed a doublet at 6.88 (2H, *d*, *J* = 8.5, H-3 and H-5) and 7.54 (2H, *d*, *J* = 8.5, H-2 and H-6), indicating B ring substitution by a chelated hydroxyl group at δ_H 13.56.

Compound **6** (derricidine) was obtained as a yellow solid, a precursor of 4-hydroxyderricidine (**5**). ^{1}H NMR spectrum of compound **6** revealed the presence of two double doublets at δ_H 7.89 and 7.59 (*J* = 16.0 Hz), characterizing the *trans*-olefinic system of chalcone. A singlet at δ_H 13.44 indicated a chelated hydroxyl group on the C-2′ position. The signals at δ_H 7.65 (2H, *m*, H-2 and H-6) and 7.44 (3H, m, H-3, H-4 and H-6) indicated an unsubstituted B ring. A prenyl group was observed via the signals at 1.81 (*s*, 3H), 1.76 (*s*, 3H), 4.57 (2H, *d*, *J* = 6.8 Hz) and 5.49, and also at 6.50 (1H, *J* = 8.4 and 2.4 Hz) with *meta* coupling at 6.48 (1H, *J* = 2.4 Hz) and *ortho* coupling at 7.83 (1H, *d*, *J* = 8.4 Hz).

Compound **7** was characterized as a trimethoxylated derivative of resveratrol (3,5,4′-trimethoxy-stilbene). It showed doublets at δ_H 7.04 and 6.91 with large *J*-coupling (16.4 Hz) characterizing trans-ethylenic chair due to two singlets of aromatic methoxyl at δ_H 3.833 (3H) and δ_H 3.832 (6H), three singlets of aromatic methoxyl and two pairs of doublets δ_H 7.45 (2H, H-2′ and H-6′) and δ_H 6.90 (2H, H-3′ and H-5′) with coupling at *ortho* (*J* = 8.4 Hz) and *meta* (*J* = 2.0 Hz), indicating one *para*-substituted aromatic system. The methoxyl groups were attributed to the 3 and 5 positions owing to two doublets with coupling *meta* at δ_H 6.65 (2H, *J* = 2.0 Hz) and one triplet at δ_H 6.37 attributed to homotopic hydrogens H-2, H-6 and to H-4, respectively.

Compounds **8** and **9** were identified in mixture. ^{1}H NMR spectrum of compound **8** (3′,4′,7-trimethoxyflavone) showed one singlet at δ_H 6.69, indicating the presence of hydrogen of a flavone C-ring. The signals at δ_H 8.11 (1H, *d*, *J* = 8.5 Hz, H-5), 6.98 (1H, *dd*, *J* = 8.5 and 2.0 Hz, H-6) and 6.98 (1H, *d*, *J* = 2.0 Hz, H-6) belong to the hydrogens H-5, H-6 and H-8 (A-ring). Chemical shifts from a B ring were observed at δ_H 7.53 (*dd*, *J* = 8.5 and 2.0, H-6′), 6.96 (*d*, *J* = 8.5, H-5′), 7.35 (*d*, *J* = 2.0, H-2′), indicating substitutions on C-3′ and C-4′ positions due to methoxyl groups at δ_H 3.95 (*s*, 3H) and 3.97 (*s*, 3H). A methoxyl group was observed at δ_H 3.92 (*s*) attributed to C-7, which was confirmed through correlations on a contour map. Combined, these spectral data allowed the identification of 3′,4′,7-trimethoxyflavone.

Compound **9** was identified as the lignan yangambin through signals at δ_H 6.57 (4H, *s*), δ_H 4.75 (2H, *d*, *J* = 4.0 Hz), 4.31 (2H, *dd*, *J* = 9.0 and 6.9 Hz), 3.92 (2H, *dd*, *J* = 9.0 and 6.9) and δ 3.09 (2H, *m*). These signals showed correlation on an HSQC contour map: δ_C 102.7 and δ_H 6.57; δ_C 85.9 and δ_H 4.75; δ_C 54.3 and δ_H 3.09 and on HMBC: δ_C 137.4 and δ_H 6.57 (3J); δ_C 137.4 and δ_H 3.82 (3J); δ_C 85.9 and δ_H 6.57 (3J); δ_C 85.9 and δ_H 4.31 (3J); δ_C 54.3 and δ_H 4.75 (2J).

In order to determine minimum inhibition concentrations (MICs), hexanic branches and CH_2Cl_2 root extracts were tested against *C. albicans*, *C. gattii* and *C. neoformans*. The only active extract was CH_2Cl_2 root, which showed an MIC of 800 μg/ml against *C. gattii*. Investigation of the compounds obtained from this extract showed that 4-hydroxylonchocarpine was active against all three species. 4-Hydroxylonchocarpine has been described as a chalcone with several biological activities, including antimicrobial, anticancer, antituberculosis, antimalarial, antioxidant and anti-inflammatory potential (Kuete et al., 2013) and, more recently, induced lactate dehydrogenase (LDH), phosphorylation of the eukaryotic elongation factor 2 (eEF2) and AMP-activated protein kinase (AMPK) and activated caspase-3 (Cursino et al., 2016).

The antifungal activity of 4-hydroxylonchocarpine showed significant results for *C. albicans*, *C. gabrata*, *Microsporum audorium* and *Trichophyton rubrum* (Mbaveng et al., 2008), and against *C. tropicalis*, *C. albicans* and *C. neoformans* (Dzoyem et al., 2013), but that for *C. gattii*, described in the current study (Table 1), is being done so for first time, as far as we know.

The properties of compound **8** (3′,4′,7-trimethoxyflavone) have already been investigated by another study, which isolated it and tested its effects on phosphorylation of eEF2, AMPK and eIF4E (Cursino et al., 2016). Compound **9** (yangambin) has been described from a wide variety of species, including *Achillea holosericea*, Asteraceae (Ahmed et al., 2002), *Magnolia fargesii*, Magnoliaceae (Kim et al., 2009), *Ocotea duckei*, Lauraceae (Antunes et al., 2006), as well as previous studies of *D. duckeana* (Cursino et al., 2016). It showed analgesic and anticancer activities (Hausott et al., 2003) and protective effect related to cardiovascular collapse (Araújo et al., 2001).

As this genus is known for the presence of prenylated flavonoids, the present results corroborate the location of the *Deguelia* within the Fabaceae. The present study describes antifungal activity of 4-hydroxylonchocarpine against *C. gattii* for the first time, which indicates a preliminary antifungal activity. Further studies, especially in the pharmacological area, are necessary to confirm these results.

Authors' contributions

NML, LMCC, ACO, JVNM, CVN and JVBS conceived and designed the experiments; NML and JVNM collected the plant sample and made herbarium exsiccates; NML, LMCC, AML, ACO and JVNM performed the experiments; NML, LMCC, AML, JVBS and CVN analyzed the data; JVBS and CVN supervised the laboratory work and contributed with reagents/materials/analysis tools, as well as to critical reading of the manuscript; NML, LMCC and CVN wrote the paper. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

All authors have none to declare.

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